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# In-Season Crop Management Effect On Soybean Yield and Grain Quality in Eastern South Dakota

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# IN- SEASON CROP MANAGEMENT EFFECT ON SOYBEAN YIELD AND GRAIN QUALITY IN EASTERN SOUTH DAKOTA

BY

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THESIS ACCEPTANCE PAGE

Kelsey Bergman

This thesis is approved as a creditable and independent investigation by a candidate for the master's degree and is acceptable for meeting the thesis requirements for this degree. Acceptance of this does not imply that the conclusions reached by the candidate are necessarily the conclusions of the major department.

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This thesis is dedicated to my family and friends for supporting me.

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# CONTENTS

# ABBREVIATIONS

| | |
|---|---|
| MG | maturity group |
| MRML | Most recent matured leaves |
| PDate | planting date |
| R1 | beginning flower |
| R2 | full flower |
| R3 | beginning pod |
| R5 | beginning seed fill |
| R6 | Seed fill |
| R7 | Beginning maturity |
| R8 | Physical maturity |
| SERF | South East Research Farm- Beresford, SD |
| V4 | four trifoliate |

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# ABSTRACT


IN-SEASON CROP MANAGEMENT EFFECT ON SOYBEAN YIELD AND GRAIN QUALITY IN EASTERN SOUTH DAKOTA

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Soybean [Glycine max (L.) Merr.] yield has increased over time by introduction of newer varieties and improved agronomic practices. However, grain protein concentration has been decreasing at the same time. New field studies in 2018 and 2019 investigated in-season crop management practices effect such as use of foliar protection application, fertilizer application, different maturity length or different planting dates on grain yield and grain quality. The studies were established near Brookings, SD and Beresford, SD. In-season treatments were targeting nutrient availability or protecting soybean canopy during the grain filling period. Such treatments included the use of fungicide, insecticide, or supplying additional nutrients through foliar applications at the beginning of grain fill of the soybeans. Nitrogen and S applications were also made at different timings either at pre-season, V4, and R3 growth stages. Biomass samples were taken at R5, R6, and R7 and partitioned into parts of the plant. Grain yield, grain protein concentration, and grain oil concentration were taken at harvest and analyzed. While foliar protection application or fertilizer treatment effects did not impact grain yield or seed composition, year, location, and maturity group often influenced these parameters. Applying what is needed for each field is important when trying to maintain grain yield and quality.

# CHAPTER 1

# INTRODUCTION

Soybeans [*Glycine max* (L.) Merr.] have been produced and harvested on nearly 36 million hectares in 2018 in the United States (USDA-NASS, 2019). Over the last century, soybean yields increased by 0.0234 Mg $ha^{-1}$ $yr^{-1}$, and with this yield gain, a slow dilution of soybean grain protein concentration occurred (Rowntree et al., 2013a). Soybeans seeds are generally composed of approximately 34 percent protein and approximately 19 percent oil at the 13% moisture content (Stowe, 2017, Kaur et al., 2017). Grain protein and oil concentrations usually are negatively correlated. Soybean pricing is only based on the yield along with grain grading quality which currently does not include seed composition such as levels of oil and protein, like for other crops. Changing grain composition has not been an issue on the soybean grain market yet, as it has in small grains, such as wheat (*Triticum aestivum* L.; Kaur et al., 2017, Weidenbenner et al., 2014). Many wheat producers receive grain price reduction because they did not meet the protein requirement. Soybean meal is one of the many uses for soybeans produced in the United States. Lower concentration levels of protein often affect meal processors trying to meet quality standards, and also impacts farmers who feed soybean meal to their animals. If protein concentration levels are lower, then the farmer will have to either supplement protein or use, and buy, more soybean meal in order to supply the same protein amount as it would be with higher grain protein concentration soybeans. Research over the years has shown that there are many factors that influence seed protein concentration levels. There are three main categories that these factors fall under; genetics, management, and the environment influenced factors also known as the G x M x E. The realized grain protein level is the result of the interactions of the three factors mentioned above.

Looking at the genetics portion, in recent years soybean protein levels have declined partially due to breeding effort; that selecting varieties with higher or high protein levels generally were lower priority due to the fact that it is easier to improve grain yield (Anthony et al., 2012). While protein and oil concentration are not specifically bred or improved due to a larger demand for higher yield, this has led to an increase in oil concentration levels over time. Yield increase also contributed to changes in soybean growth; research comparing historical varieties documented that modern soybean varieties spend more time in reproductive growth allowing longer time to develop pods and seeds than older varieties (Rowntree et al., 2013b). Knowing when to select varieties that are known to be less susceptible to pathogens, or certain pests can help maintain foliar canopies through the growing season. While selecting for genetics is important for increasing yield and grain quality, the environment is vital as well.

The environment plays a key role in seed quality and development through weather related events. High air temperatures, and moderate to low amounts of rainfall during the seed filling period and during the reproductive growth generally result in higher protein concentration in soybean seeds (Rotundo and Westgate, 2009). Increased temperatures and high amounts of precipitation are said to increase yield (Rowntree et al., 2013a). The air temperature effect on grain oil concentration showed mixed results; Rowntree et al, (2013a) documented oil concentration increase with lower air temperatures, while Anthony et al. (2012) with higher air temperature, especially if these temperatures occur after the R5 (beginning seed) growth stage (Naeve and Huerd, 2008, Kaur et al., 2017,). Drought conditions during late reproductive stages have been found to decrease grain protein concentration in the seeds (Rotundo and Westgate, 2009). The magnitude of the increase in protein synthesis level depends on the timing and extent of the environmental stress. Seed mass also can be heavily influenced by environmental factors such as water availability and pest pressure. Stress during seed fill will have the most abundant increase in protein (Naeve and Huerd, 2008). This increase in protein could be because the seed size decreases and could be why protein is not normally correlated with higher yield (Rotundo and Westgate, 2009). Seeds at lower parts of the plant tend to have higher amounts of oil, while at the upper parts of the plant have more protein because the accumulation of oil in seeds often starts earlier in seed development than protein (Rotundo and Westgate, 2009; Saldivar et al., 2011, Huber et al. 2016). With greater amounts of N, there is likely to be more protein formed in the seeds (Rotundo and Westgate, 2009). If rain is abundant early in the growing season, there would be an increase in N leaching in the soil making it unavailable to the soybean later in the growing season. Along with temperature and water stress, other weather events that can remove or damage leaves can have an impact on photosynthesis and protein synthesis, such as hail. Hail damage can also lead to foliar disease development later in the growing season. Foliar diseases can reduce seed mass when not treated (Weidenbenner et al., 2014). Disease management through fungicide applications can help reduce soybean pathogens during the season.

Though a producer has minimal control over the weather conditions during the grain fill period, there is some ability to influence the timing and length of the grain fill period through the choice of planting date and the maturity length of the planted variety.

Previous research has shown that planting date can have a major impact on soybean protein levels. When it comes to planting soybeans, later planted soybeans tend to have a higher protein concentration then earlier planted soybeans (Jaureguy et al., 2013, Rowntree et al., 2013a). When planted earlier in the growing season, the soybeans will have longer time before flowering. When planted later, the soybeans have to mature faster. Often times, there is much shorter time difference in soybean flowering time than the time difference between planting in early and delayed planted soybeans, as soybean flowering is partially triggered by the day length time following the summer solstice. Length of the growing season forces certain parts of the country, like South Dakota, to plant shorter maturity groups of soybeans to enable them to reach physiological maturity due to generally later planting dates and an earlier typical first frost dates compared to growing environments further south. Finding the right maturity group for the area is key to reaching full yield potential and maintain grain quality. Just like the maturity group, nutrient applications are another management practice that can influence grain protein levels. Soybean crops normally derive between 60 and 89 percent of their total N uptake from N fixation (Abendroth et al., 2006; Tien et al., 2002). However, more conservative estimates suggest that the contribution of N fixation can meet from 25 to 75 percent of total plant N demand (Deibert et al., 1979; Salvagiotti et al., 2008; Collino et al., 2015). Protein synthesis is the process of forming amino acids into proteins. Nitrogen is one of the major components of these amino acids that are the building blocks of proteins (Galili and Galini, 2008). Nitrogen and S tend to interact at the metabolic level in such a way that an imbalance in their supply reduces the crop yield (Jamal et al., 2005). According to

Jamal et al. (2005), the inclusion of S fertilizer recommendation (up to 20 kg S $ha^{-1}$) for optimum soybean yield in S-deficient soils is necessary.

Protecting the canopy through management practices (e.g. supplying nutrient, or protection against pests) can prolong leaf senescence. By delaying leaf senescence and maintaining photosynthesis, soybean plants will have a longer time to produce protein in the seeds (Garcia and Hanway, 1976). Protein development starts much later then oil synthesis and by extending physiological maturity will help increase protein concentrations in the seeds (Saldivar et al., 2011, Huber et al. 2016). Adding inoculum during planting is another way to potentially increase protein. Abel and Erdman (1964) and Caldwell and Vest (1970) state that inoculation with *Bradyrhizobium japonicum* successfully increase soybean nodulation with increases in plant fresh weight, seed yield, and protein concentration in soils with a low or absent native population. "*B. japonicum* improved plant development and growth and the grain and protein yields of soybean crops." Co-inoculation with *Azospirillum spp*. has also been successful in increasing root density and length, root biomass, root hair development, shoot biomass, nodule number, and the fresh weight of soybeans" (Molla et al. 2001). Management practices are potentially the easiest ways to increase yield and maintain the percent of protein in the seed.

Yield is the main determining factor to determine income, along with market price. Since there is no economic incentive for seed oil or protein level at the grain elevator, it is harder to convince farmers to produce a crop that has higher seed protein (or better seed composition). The main question is, what can be done to maintain protein levels while still increasing yield?

Investigation of these management practices that could influence grain quality led to a multi-state collaborative research project. The goal of this project is to identify management factors affecting soybean seed protein and its composition, and to define the best management practices for increasing soybean seed quality. To accomplish this goal, field studies were established spanning across the Midwest to provide new information on how to increase soybean seed protein and essential amino acids by implementation of specific cultural practices. Field experiments were targeting one of the following components of management decisions: 1) altering growing environment conditions during seed filling (SF) using different maturity groups, and different planting dates, 2) altering nutritional status of the plant with applications of N and S at different timings of application (pre-plant, early or late growing season) or different fertilizer rates, or 3) maintaining the plant health through protection of leaf, extending leaf retention, and delaying nutrient remobilization. In South Dakota, objective of the field experiments were to 1) determine the effects of foliar protection applications at beginning of seed filling on protein levels in soybean seeds in different maturity group varieties, and 2a) determine the effects of N and S application rate and timing on protein levels in soybean seeds in different maturity group varieties, and 2b) different planting dates.

# CHAPTER 2.

# FOLIAR PROTECTION EFFECT ON SEED COMPOSITION AND CANOPY RETENTION

## 2.1 INTRODUCTION

Foliar protection applications done in-season can help maintain canopies. Canopy research determined that increase in light interception with narrow rows spacing for soybeans during grain filling strongly correlated with the grain yield increase (Board et al., 1990; Bullock et al., 1998; and Egli, 1994). An increase in aboveground biomass, light interception, and assimilate utilization are all contributed to yield increases in narrow rows compared to wide-rows production system (Andrade et al., 2002, Board et al., 1990; Bullock et al., 1998; and Egli, 1994).

Foliar diseases or insects can reduce leaf area and reduced leaf area will lower photosynthesis, and reduce grain yield (Bassanezi et al., 2001). Foliar protection applications can aid in maintaining healthy crop canopies during grain fill period, which relieves crop stress and extends photosynthetic production. Fungicides are often marketed as the “cure all” for soybean diseases, but have been shown to mainly benefit fields that have a problem/disease present (Jordan, 2010).

If foliar diseases or insects become a problem within a field early in the growing season, then the soybeans express their stress by reduction in canopy height, and the failure of closing the canopy between rows (Hansen et al., 2003). Typical preventive pesticide application timing recommended around the early reproductive stages (either due to insects presence or probability of pathogen infection and prevention) which also coincide when canopies start to close, to prevent major losses and keep stress levels to a minimum. In South Dakota, white mold (*Sclerotinia sclerotiorum)* is a disease that can cause severe yield losses. One of the management practices to help reduce the amount of white mold in a field is by increasing row spacing (typically .75 meter) or reducing plant population to lower relative humidity in the crop canopy. Row spacing also affects light interception in the lower portion of the soybean canopy. With the wider row spacing there is more light that can reach the lower parts of the soybean plant and the ground which will increase the air temperatures within the canopy during seed-fill due to decreased albedo effect. This effect has shown to result in higher soybean protein concentrations (Naeve and Huerd, 2008). There is little evidence of yield increases in soybean production with foliar fungicide application, however, it does help maintain yield when disease is present in the field. According to Jordan (2010), a yield increase is not seen when diseases or insects are not present. Foliar protection applications directly help maintain yield potential when there are problems in the growing season. The yield benefit from fungicide applications has been attributed to maintaining healthier plants that can function better compared to the untreated control and infected plants (Badenoch-Jones et al., 1996; Bryson et al., 2000; Grossmann et al., 1999; Grossmann and Retzlaf, 1997; Kuroda et al., 1996). Increasing seed mass while maintaining seed number could explain how fungicides have contributed to grain yield in some environments but not others (Weidenbenner et al., 2014). Maintaining healthy canopy through the early reproductive stages can help to achieve the crop’s yield potential.

Those who use fungicides are those who most commonly found a problem in their fields or have a recurring problem. Wrather and Koenning (2006) found that foliar diseases accounted for only about 7.2 percent of total yield reduction over the three-year period of their study in 2005. Other publication however, has shown that fungicide applications are profitable even when disease problems are not present (Orlowski et al. 2016). However, following the integrated pest management principles, fungicide application should only be used when there are known or anticipated problems in the field to manage fungicide resistance development.

Another way to help maintain healthy canopies is by preventing nutrient deficiencies. Foliar fertilizers sometimes contribute to observed yield differences (Jordan, 2010). These nutrient deficiencies could be from lack of fertilization before planting, not supplying adequate amount of the nutrient that the crop needs or plants not being able to acquire it from the soil. Garcia and Hanway (1976) speculated that minimizing the nutrient depletion of soybean leaves caused by remobilization was the cause of yield increases from foliar fertilization. They hypothesized that this reduction in nutrient depletion delayed senescence of the soybean and extended the leaf photosynthetic activity and improved seed fill. A study by Rotundo and Westgate (2009) showed fewer seeds per plant resulted in greater availability of nitrogen per seed that yielded high protein concentration seeds. When there are fewer seeds per plant, yield is often reduced (Rees et al., 2019). The objective of this project is to determine the effects of foliar protection applications at the beginning of seed filling on protein levels in soybean seeds in different maturity group varieties.

## 2.2 MATERIAL AND METHODS

### 2.2.1 Site description and experimental design

This study was conducted in the eastern part of South Dakota at two of the South Dakota State University Research Farms; near Brookings, SD (44.3114° N, 96.7984° W), and at the Southeast Research Farm (SERF; 43.0805° N, 96.7737° W) near Beresford, SD. The soil types were Divide (fine-loamy over sandy, mixed, superactive, frigid Aeric Calciaquolls), and Egan-Wentworth complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at Brookings and Egan-Clarno-Tetonka complex, which is a fine-loamy/fine-silty, mixed, superactive, mesic Typic Haplustolls; Egan-Clarno-Trent complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at SERF in 2018 and 2019, repsectively.

Two soybean varieties, GH1024X, GH2041X (maturity group 1.0 and 2.0 respectively, Golden Harvest Seed, Minnetonka, MN) were planted in this research. Six different foliar protection treatments were applied on each variety at R3 (beginning pod) growth stage: un-treated control, fungicide only, insecticide only, foliar fertilizer only, fungicide plus insecticide, and a combination of fungicide, insecticide, and foliar fertilizer. Plots received the following products according to treatment assignment: Trivapro [Benzovindiflupyr 2.9%, Azoxystrobin 10.5%, Propiconazole 11.9%, Group 11, 3(DeMethylation Inhibitors), 7(Succinate dehydrogenase inhibitors)] fungicide at 0.172L ha$^{-1}$; and Miravis [Pydiflumetofen 18.3%, Group 7] fungicide at 0.172L ha$^{-1}$; Endigo [Lambda-Cyhalothrin 9.48%, Thiamethoxam 12.6%, Group 3A, 4A; demethylation inhibitors and phenlamides)] insecticide at 0.052 L ha$^{-1}$, and Generate (0.28% Fe, 0.11%

Mn, 0.14% Cu, 0.11% Zn, 0.001% Mo, 0.52% Co, 0.11% Na) foliar fertilizer at 0.366L $ha^{-1}$ rates when sprayed at 140 L $ha^{-1}$. The combination of variety and foliar product treatments were arranged in a randomized complete block design.

Corn (*Zea mays L.*) was the preceding crop at both locations in 2019 and the Brookings site in 2018. These sites utilized conventional tillage practices and prior to planting fields were field cultivated, while at Beresford in 2018 soybeans were planted in no-till ground following oats (*Avena sativa* L.). In 2019, soybeans at the Beresford site required replanting due to very poor plant stand caused by wet weather conditions during planting and emergence window. Soybean varieties were planted at 346,000 seeds $ha^{-1}$ in 76-cm rows (Table 2-1). Plots were maintain weed free. Plots sizes were 3m x 18.3m in Brookings and 4.5m x 13.7m at SERF in 2018 and at both locations in 2019.

2.2.2 Field data collection

Before planting, soil samples were taken from each replication (15 cores of 2 cm diameter in each replication) and separated to 0-15cm, 15-60cm; soil samples were air dried with forced air then ground to pass through a 2 mm sieve, and sent off to a certified commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient concentrations, soil pH and organic matter (OM). The laboratory used the 1:1 soil-water slurry method for soil pH, the loss of ignition method for OM, the cadmium reduction method for $NO_3^-$-N and the monocalcium phosphate method for $SO_4^{2-}$—S concentration determination (AgSource Laboratories, 2020).

At both locations, the foliar treatments were applied at the R3 growth stage in end of July 2018 and 2019 (Table 2-1). In 2018, at the SERF site the foliar fertilizer and the combination of all product treatments were discarded, due to an initial planting error that caused these foliar treatments to apply on the same maturity group treatment resulting 8 plots, all the same maturity group variety, with the same foliar treatment (the switch of maturity groups between treatments was noticed only at the beginning of leaf senescence). Visual plant damage ratings were taken before foliar application, 14, and 28 days after application for extent of leaf damage and presence and extent of disease based on percentage of leaf damage and percentage of the plant they made up.

After emergence (around V3 growth stage) and at physiological maturity, plant population was estimated through stand counts at four locations in the center rows within each plot. The number of plants were counted on one meter length of a row and plant population were calculated from.

At R5 (beginning seed), R6 (full seed), and R7 (beginning maturity) growth stages biomass samples were taken from 0.5 meter section of a non-border row. Sampling times presented in Table 2-1. The R5 and R6 samples were partitioned into leaves, stems, branches (including petioles), pods, and fallen leaves. The R7 samples were partitioned into leaves, stems, branches (including petioles), pod shells, seeds, and fallen leaves. Fallen leaves were any leaves or petioles that fell in the area biomass was collected from (0.38 m from each side of the row). All samples were dried at 60 $^{\circ}$C until constant weight and the dry biomass accumulation was calculated.

Grain harvest was done in late October (Table 2-1). The middle two rows were harvested with a Massey Ferguson 8XP plot combine. Grain weight, grain moisture and test weight readings were recorded. Plot lengths were measured to determine grain yield.

Grain protein and oil concentrations were determined with InfraTec Nova (FOSS Analytics, Hillerød, Denmark) instrument. Seed mass (200-seed weight) were estimated by weighing grain samples collected from the harvested plots. Grain yield, grain protein and oil, and the 200 seed weight data was adjusted to 130 g $kg^{-1}$ moisture content.

### 2.2.3 Statistical analysis

Results are analyzed in R Studio in ANOVA and Fishers-LSD at 0.05 significance level. The foliar application treatments, maturity group, year, and location, were all considered fixed factors in the statistical model. The SERF site in 2018 was analyzed separately due to the planting and foliar application error. The other three site years were combined for statistical analysis.

## 2.3 RESULTS

### 2.3.1 Weather

Daily minimum and maximum temperatures, and precipitation in 2018 and 2019 for each site are shown in Figure 2-1 and monthly mean minimum and maximum temperatures and monthly precipitation are presented in Table 2-2. Average highs temperatures ranged from 11.0 to 28.89 °C while mean low temperatures ranged from 0.6 to 17.87 °C in the May - October period.

Monthly total precipitation data were also calculated. From May until October rainfall was ranging from 19 to 221 millimeters a month. Precipitation was higher in 2018 at the SERF site. SERF also had higher precipitation early in the 2019 season. The largest monthly precipitation was recorded in 2019 September at the Brookings site with

220.98mm among the four site-years. Brookings also had the largest amount of precipitation in one day (159.3mm) which occurred in July 2018 in Brookings. Each site received above normal precipitation from planting until harvest (e.g. the SERF site in 2018 was nearly doubled the 30 years normal).

### 2.3.2 Field Characteristics

Soil samples taken before planting and analyzed for pH, OM, N, S are shown in Table 2-3. Soil pH ranged from 5.7 to 7.6 in the 0-15cm soil layer and from 6.5 to 7.6 in the 15-60 cm depth. Organic matter content in the top 15 cm depth ranged from 3.2% to nearly 4.5% while in the 15-60 cm soil depth it ranged from 2.6 to 3.0% among the sites. Soil $NO_3^-$-N ranged between 2.5 and 22.5ppm, and between 1.0 and 8.0 ppm, while $SO_4^{2-}$-S ranged between 3.0 and 11.3 ppm and between 2.0 and 8.8 ppm in the 0-15 cm and 15-60 cm soil depths, respectively (Table 2-3).

### 2.3.3 Stand counts

There were no statistical differences in early season or final plant stand among the foliar treatments (Table 2-4 and Table 2-5). However, the ANOVA analysis detected statistical differences between the MGs as main factors and the year x location interaction both for early-season and final stand counts (Table 2-4). While plant stands were statistically different between the MGs, the differences was 12-13,000 plants ha$^{-1}$ across the site years, and treatments at either time (Table 2-5). The 2019 SERF site had approximately 21,000 plants ha$^{-1}$ smaller plant stand at the early part of the growing season averaged across the other factors (Table 2-5).

At the SERF site in 2018 early season plant stand did not differ, while the final plant stand was approximately 6,000 plants $ha^{-1}$ larger in MG2 variety plots averaged across the foliar treatments (Table 2-7).

2.3.4 Grain yield, protein, and oil concentrations

Both grain yield, grain protein and oil concentration were influenced by the interaction of growing season and the location (Table 2-4). However, only the grain yield and the grain oil concentration differed due to the MG, and to the year x location x MG interaction (Table 2-4). The foliar application treatments did not have yield differences in grain yield, grain protein concentration or grain oil concentration (Table 2-4). Only a few significant interactions were detected among the other interactions and their impact on grain yield and grain components were inconsistent (Table 2-4). For example, the location x MG x foliar application treatment influenced the grain yield, while the year x MG and year x location x foliar application treatment influenced the grain oil concentration at the p=0.05 significance level (Table 2-4). Data on grain yield, grain protein, and oil concentrations are shown in Table 2-5. Maturity group 2 variety out yielded MG 1 variety by about 0.50 Mg $ha^{-1}$ across the foliar treatments and site years. Oil concentration was also numerically higher with fungicide and insecticide treatment compared to the other foliar treatments averaged across the maturity groups and site years. Brookings MG1 had the significantly highest protein (0.8 percent higher) while all other MG in Brookings and SERF were significantly higher in oil (0.4 percent higher) this could be why we saw the year by location by treatment interaction (Table 2-6). 2019 at SERF numerically was higher than Brookings in both years and fungicide and treatments with fungicide tend to be the highest yielding (Table 2-6).

Yield in 2018 at Brookings MG2 was the highest than all other treatments in the year by location by MG interaction. Protein concentration was the highest in MG1 variety in Brookings across the foliar treatment and oil concentrations was the opposite with Brookings MG1 being the lowest (Table 2-6). At the Brookings sites the MGs differed in grain protein and oil concentrations, while at the SERF site the MGs had similar grain composition.

The SERF site in 2018 that was analyzed separately (Table 2-7) fungicide and treatments that had fungicide tended be significantly the highest yielding. MG2 was significantly higher in grain yield, grain protein concentration, and grain oil concentration (Table 2-7).

2.3.5 Biomass accumulation and partitioning

Biomass partitions did not differ among foliar application treatments at either growth stages across the three site-years (Table 2-8). There were no significant differences among the treatments at R5 growth stage in any part of the plant, but the year by location interaction was significant (Table 2-8). Soybean plants tended to have the largest amount of biomass when the plant was at R6; this is before they start dropping their leaves. Biomass in 2019 was lower approximately by ~1000kg $ha^{-1}$ at both locations and at all three growth stages than in 2018 (Figure 2-2). Treatments did not change the amount of biomass that the plant maintains through the late reproductive (R5-R7) stages except for the leaves with the foliar fertilizer treatment at R6 growth stage which was lower than the foliar fertilizer, fungicide, insecticide combination, but did not differ from the untreated control (Table 2-9). Only the leaves biomass differed at the R6 growth stage, while the leaves, and pods biomass accumulation differed at the R7 growth stage

(Table 2-8). Leaves with either fungicide only or combination but containing fungicide treatments had the three largest leaves biomass across MGs and site years (Table 2-9). Only the fungicide and insecticide treatment did not differ from the other three non-fungicide containing treatments. At the R5 and R6 growth stages, MG1 variety's stems, were higher in biomass, while the branches were higher in MG2 (Table 2-10). R7 stems were significantly higher in MG1 while the rest of the partitions, branches, pod shells, seeds were higher in MG2 (Table 2-10). R5 and R7 leaves, stems, and branches were the highest in 2018 at Brookings, while the R5 pods were significantly higher in 2019 Brookings, and the R7 seeds and pod shells were higher in 2019 at the SERF in the year by location interactions (Table 2-10). All R6 biomass partitions and total biomass accumulation were significantly higher in 2018 Brookings compared to the other site years. Table 2-11 shows the proportional distribution of the plant partitions relative to the total biomass.

Over time, pod/seed biomass increased while other parts of the plant biomass decreased due to resources being moved to into the pods and away from the leaves and stems.

2.3.6 Disease and pest damage assessment

There were some seasonal differences in the pre application pest ratings comparing between 2018 and 2019. In 2018, initial pest damage ratings were (more leaf damage) higher prior to the R3 spraying then in 2019. There was no significant differences between the years in diseases pressure pre application. After application, there was higher insect damage in the untreated control and the foliar fertilizer only treatments at both locations. Fungicide treatment also had more insect damage at

Brookings in both years. However, no noticeable disease pressure differences were observed 14 to 28 days after application. (Table 2-12, 2-13)

## 2.4 DISCUSSION

### 2.4.1 Weather

Negative monthly mean temperatures only occurred in April 2018 at both sites, and only reached subzero temperatures on May 10, 2019 at the SERF. These early spring low temperatures prevent growers from early planting (generally planting does not occur in April in South Dakota) and achieving a higher soybean grain yield. In addition, April 25th is the first date that soybeans can be planted in South Dakota and be protected by crop insurance. Large amounts of rainfall could affect plant stand during the season which would lead to less yield. Rotundo and Westgate (2009) found protein increases when there is water stress (lack of water) during grain fill. While none of these site years had these type of water stress, at the Brookings site in 2018 had some of the lowest amounts of precipitation (still higher than the 30 year average) during the late reproductive stages, during the two growing seasons and had higher levels of protein then in 2019. At SERF in 2018, the trial had large amount of rainfall during grain fill and warmer weather throughout the growing season and ended up having the highest yield and protein concentration in this studying agreeing with the findings of Rotundo and Westgate (2009).

### 2.4.2 Grain yield, and quality

The location effect on yield is related to environmental factors such as the higher early season precipitation and warmer temperatures (Naeve and Huerd, 2008) during the growing season at the SERF site.

Picking a MG that is right for each location is important when trying to maximize the light interception which will influence yield. Brookings does not commonly plant MG2 due to how far north it is (Hall et al. 2012).  Though unlike the nitrogen and sulfur study (Chapter 3), the MG2 variety did better than the MG1. The MG2 variety takes a longer time to mature allowing the leaves to stay on longer than the MG1 varieties. Because of this, the yield and protein levels benefited.

South Dakota has a problem with white mold in soybeans. One of the fungicides used in this study was targeted to control white mold. However, the R3 application timing is late to prevent white mold infection, but the grain filling period was the main focus of this study. Future studies could address the effect of different fungicide (or plant protection product) application timings effects on grain quality and disease development and their interaction. There were no statistical differences in disease pressure in the 14 and 28 days after application ratings. There could have been more differences later in the season that were not evaluated.

Previous research has found that applications of fungicide are profitable even when disease problems are not shown (Orlowski et al., 20016). However, this study did not show yield benefit in relatively low disease pressure conditions. In addition, spraying fungicide and insecticide below the economical threshold of the pest (e.g. not following the integrated pest management approach) can accelerate the resistance development problems.

2.4.3 Biomass

Biomass was collected at the R5, R6, and R7 growth stages. Some of the biomass weights differences occurred may be because the timing of sample collection may not have happened at the exact plant developmental stages due to the large amount of plots that needed to collected and partitioned during the grain filling period. The two MG samples were collected at the same time at the R5 growth stage while in later stages they were collected separately when the different MGs reach the target growth stage. The same sampling time can explain biomass accumulation differences between MGs at the R5 growth stage.

Branches and petioles partitions were collected together and could be why there was a dramatic decrease from 1210 to 714kg $ha^{-1}$ when going from R6 to R7 growth stage as the petioles fall from the plant. In 2018, there was lodging in the fields which also caused some difficulties to precisely define the area and collect the fallen leaves and petioles which can explain the larger decline in total biomass from R6 to R7 growth stages in that year. Fungicide application delayed leaf senescence which resulted leaf biomass differences at R7 growth stage, similarly to Parker and Boswell (1980) observations.

Both growing seasons had above normal precipitation, which affected the stand uniformity, even though the plant stand did not substantially differ among the treatments. However, the not completely uniform plant stand could vary the number of plants collected in a sample (plants were cut from a 0.5 m section of the row) impacting total biomass accumulation and even the partitioning of the biomass. When a lower number of plant was collected, the soybean plants had more branches which meant that there were more pods per plant. These plants also tended to have more leaves due to the number of branches. Planting time can also affect how many pods a plant has. Planting occurred at close to optimal time in the season in 2018 and had a longer time before the summer solstice, which leads to a longer amount of time that the plant can grow and produce nodes. Higher numbers of nodes leads to a larger number of pods per plant.

## 2.5 CONCLUSION

Foliar protection application at the beginning of the grain fill period generally did not impact grain yield or grain protein in our four site years. While we did not see a common trend in our findings, we had very low insect and disease pressure at each site year. This was also coupled with fairly challenging growing seasons during the two years of the study, including a wet growing season with delayed planting in 2019. Even though leaf senescence was somewhat delayed with fungicide applications, overall biomass accumulation or partitioning was not affected by the in-season crop management treatments, nor was grain yield. With currently no incentive on the grain market for the higher protein content, the likelihood of foliar applications used without pest pressure is unlikely to become adapted as a common practice to increase protein levels. Along with this is the thought of resistance development becoming more prevalent. If a field was found to reach economical threshold of insect pressure or prone to disease problems year after year, applying fungicide and/or insecticide could lead to maintaining higher grain yield levels or increase yield compared to the untreated control, and potentially can help to increase grain protein levels as well.

In the future, finding areas that have been known to have pest/disease presence would be important to find out if these foliar applications would work more consistently.

Also, applying fungicides at different timings, or using different fungicides and insecticides could change some of the findings. Delaying disease and insect damage ratings following the fungicide application would be important to see if there would be a significant change in protein concentration and yield. Analyzing nutrient composition of the biomass collected would be the short term goal to see if by applying these foliar treatments affected the nutrient composition in each or either part of the plant.

**Table 2-1 Experimental design, varieties used and other crop production parameters, along with planting, treatment application, biomass and machine harvest dates.**

| | 2018 | | 2019 | |
|---|---|---|---|---|
| **Field Activities** | **Brookings** | **SERF** | **Brookings** | **SERF** |
| **Varieties** | GH1024X, GH2041X | | | |
| **Experimental Design** | RCBD | | | |
| **Row spacing** | 0.76m | | | |
| **Replications** | 4 | | | |
| **Seeding Rate** | 346,000 viable seeds $ha^{-1}$ | | | |
| **Plot dimensions** | 3m x 18.3m | 4.5m x 13.7m | | |
| **Tillage** | Conventional | No-till | Conventional | |
| **Rotation** | Corn-Soybean | Oat-Soybean | Corn-Soybean | |
| **Planting Date** | May 15 † | May 17 | June 2 | June 8 |
| **R3 application** | July 21 | July 20 | July 25 | July 25 |
| **R5 Biomass** | Aug. 9<br>Aug. 13 | Aug. 6 | Aug. 13<br>Aug. 20 | Aug. 15<br>Aug. 19 |
| **R6 Biomass** | Aug. 30<br>Sep. 7 | Aug. 20<br>Aug. 23 | Sep. 4<br>Sep. 10 | Sep. 3<br>Sep. 5 |
| **R7 Biomass** | Sep. 14<br>Sep. 19 | Sep.5<br>Sep.12 | Sep. 23<br>Oct. 4 | Sep. 23<br>Sep. 30 |
| **Machine harvest** | Oct. 19 | Oct. 18 | Oct. 19-20 | Oct. 18 |

† multiple dates for a field activity indicate that the MG1 and MG2 varieties have been sampled on separately on the days presented

**Table 2-2. Monthly mean minimum and maximum air temperature and monthly precipitation accumulation near Brookings and Beresford, SD in 2018 and 2019.**

| | ----2018 Brookings---- | | | -----2018 SERF----- | | | ----2019 Brookings---- | | | ----2019 SERF---- | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Mean Max | Mean Min | Precip. | Mean Max | Mean Min | Precip. | Mean Max | Mean Min | Precip. | Mean Max | Mean Min | Precip. |
| | ----ºC---- | | mm | ---- ºC ---- | | mm | ---- ºC ---- | | mm | ----ºC---- | | mm |
| **April** | 6.8 | -3.2 | 19.3 | 9.8 | -2.7 | 45.5 | N/A | N/A | N/A | 14.1 | 2.1 | 88.7 |
| **May** | 23.8 | 11.1 | 18.8 | 24.6 | 12.1 | 94.5 | N/A | N/A | N/A | 18.5 | 7.0 | 156.7 |
| **June** | 26.1 | 16.4 | 102.1 | 28.0 | 17.4 | 209.3 | 26.5 | 13.9 | 70.4 | 27.0 | 14.4 | 98.3 |
| **July** | 26.9 | 15.8 | 215.7 | 28.6 | 16.4 | 77.7 | 28.0 | 16.5 | 192.0 | 28.9 | 17.9 | 109.0 |
| **August** | 26.1 | 15.3 | 86.9 | 26.8 | 15.4 | 96.5 | 25.1 | 14.0 | 109.5 | 26.6 | 15.1 | 82.3 |
| **September** | 21.6 | 11.8 | 108.0 | 23.2 | 12.5 | 172.5 | 23.2 | 12.6 | 221.0 | 25.2 | 13.7 | 74.2 |
| **October** | 11.2 | 0.6 | 46.0 | 13.1 | 1.1 | 51.6 | 11.0 | 1.9 | 45.2 | 13.4 | 2.0 | 82.0 |

**Table 2-3 Pre plant field characteristics of soil pH, organic matter (OM), and soil nitrate-N ($NO_3^-$-N), and sulfate-S ($SO_4^{2-}$-S) concentration in the 0-15cm and 15-60cm soil zones in 2018 and 2019 near Brookings and Beresford, SD.**

| | Soil depth (cm) | pH | OM (%) | $NO_3^-$-N (ppm) | $SO_4^{2-}$-S (ppm) |
|---|---|---|---|---|---|
| **2018 Brookings** | 0-15 | 6.58 ± 0.35 | 4.45 ± 0.39 | 22.50 ± 7.54 | 11.25 ± 0.96 |
| | 15-60 | 7.60 ± 0.43 | 2.73 ± 0.08 | 8.00 ± 3.16 | 8.75 ± 0.50 |
| **2018 SERF** | 0-15 | 6.28 ± 0.88 | 3.38 ± 0.48 | 4.00 ± 2.58 | 7.00 ± 4.00 |
| | 15-60 | 6.88 ± 0.71 | 3.00 ± 0.47 | 5.25 ± 1.26 | 7.50 ± 2.52 |
| **2019 Brookings** | 0-15 | 5.73 ± 0.16 | 3.33 ± 0.32 | 2.50 ± 0.58 | 3.00 ± 0.00 |
| | 15-60 | 6.53 ± 0.09 | 2.75 ± 0.24 | 2.75 ± 0.50 | 2.00 ± 0.00 |
| **2019 SERF** | 0-15 | 6.20 ± 0.16 | 3.20 ± 0.00 | 2.63 ± 0.22 | 5.00 ± 1.41 |
| | 15-60 | 7.63 ± 0.45 | 2.63 ± 0.22 | 1.00 ± 0.50 | 5.25 ± 1.50 |

**Table 2-4. Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in eastern SD in 2018 and 2019.**

| | Plant Stand | | Grain | Grain Protein | Grain Oil |
|---|---|---|---|---|---|
| | Early | Late | Yield | concentration | concentration |
| **Year** | **0.007** | **<.0001** | **<.0001** | **<.0001** | 0.98 |
| **Location** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **MG** | **0.04** | **0.002** | **<.0001** | 0.40 | **<.0001** |
| **Treatment** | 0.67 | 0.74 | 0.46 | 0.24 | 0.16 |
| **Year*Location** | **0.01** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Year*MG** | 0.22 | 0.59 | 0.73 | 0.91 | **0.001** |
| **Location*MG** | 0.54 | 0.95 | 0.12 | **<.0001** | **0.0005** |
| **Year* Treatment** | 0.58 | 0.33 | 0.97 | 0.57 | 0.53 |
| **Location* Treatment** | 0.84 | 0.53 | 0.74 | *0.05* | 0.55 |
| **MG* Treatment** | 0.92 | *0.05* | 0.46 | 0.96 | 0.76 |
| **Year*Location*MG** | 0.29 | *0.09* | **0.02** | 0.19 | **<.0001** |
| **Year*Location* Treatment** | 0.97 | 0.17 | 0.82 | 0.15 | **0.02** |
| **Year*MG* Treatment** | 0.97 | 0.98 | 0.64 | 0.81 | 0.62 |
| **Location*MG* Treatment** | 0.34 | 0.52 | 0.67 | 0.46 | 0.55 |
| **Year*Location*MG*Treatment** | 0.87 | 0.65 | 0.87 | 0.565 | 0.32 |

**Table 2-5. Main treatment effect on early season and final plant stands in 2018 and 2019 near Brookings and Beresford, SD**

| | Early season | Final stand | Grain yield | Grain Protein concentration | Grain Oil concentration |
|---|---|---|---|---|---|
| | (plants $ha^{-1}$) | | Mg $ha^{-1}$ | % | |
| ***Foliar application treatment*** | | | | | |
| **Control** | 270,600 | 245,900 | 4.56 | 35.2 | 17.9 |
| **Fungicide** | 266,400 | 240,900 | 3.95 | 35.2 | 17.9 |
| **Insecticide** | 268,100 | 243,100 | 4.57 | 35.0 | 18.0 |
| **Fungicide and Insecticide** | 266,600 | 240,700 | 4.46 | 35.0 | 18.1 |
| **Foliar Fertilizer** | 266,900 | 251,100 | 5.17 | 35.1 | 18.0 |
| **FFI**[††] | 278,700 | 242,700 | 4.35 | 35.1 | 18.0 |
| ***Maturity Group*** | | | | | |
| **MG1** | 273,800a | 250,100a | 4.44b | 35.3 | 17.8b |
| **MG2** | 265,100b | 237,700b | 4.58a | 35.2 | 18.1a |
| ***Year*Location*** | | | | | |
| **2018 Brookings** | 275,300a | 196, 207c | 4.51a | 36.5a | 17.9b |
| **2019 Brookings** | 274,200a | 272,500a | 3.59b | 34.6b | 17.8b |
| **2019 SERF** | 253,300b | 254,500b | 3.94ab | 34.7b | 18.1a |

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level
[††] Combination of foliar fertilizer, fungicides and insecticide application

**Table 2-6. Interactions for grain yield, protein and oil concentrations.**

| | | Grain yield Mg ha$^{-1}$ | Grain protein concentrations | Grain oil concentrations |
|---|---|---|---|---|
| | | | % | |
| ***Year*Location*MG*** | | | | |
| **2018 Brookings MG1** | | 4.4b[†] | | |
| **2018 Brookings MG2** | | 4.8a | | |
| **2019 SERF MG1** | | 3.7c | | |
| **2019 SERF MG2** | | 4.2b | | |
| **2019 Brookings MG1** | | 3.4d | | |
| **2019 Brookings MG2** | | 3.8c | | |
| ***Location*MG*** | | | | |
| **Brookings MG1** | | | 35.7a | 17.6b |
| **Brookings MG2** | | | 35.3b | 18.1a |
| **SERF MG1** | | | 34.5c | 18.0a |
| **SERF MG2** | | | 34.9bc | 18.1a |
| ***Year*Location*Treatment*** | | | | |
| **2018 Brookings** | Control | | | 17.8abc |
| | Fungicide | | | 17.8abc |
| | Insecticide | | | 17.9abc |
| | Fungicide and Insecticide | | | 18.2a |
| | Foliar Fertilizer | | | 17.9abc |
| | FFI[††] | | | 18.0abc |

**Table 2-6 cont.**

| | | Grain yield Mg ha$^{-1}$ | Grain protein concentrations % | Grain oil concentrations |
|---|---|---|---|---|
| **2019 Brookings** | Control | | | 17.8bc |
| | Fungicide | | | 17.9abc |
| | Insecticide | | | 17.8bc |
| | Fungicide and Insecticide | | | 17.8bc |
| | Foliar Fertilizer | | | 17.9abc |
| | FFI | | | 17.8abc |
| **2019 SERF** | Control | | | 18.2a |
| | Fungicide | | | 18.0abc |
| | Insecticide | | | 18.1abc |
| | Fungicide and Insecticide | | | 18.2ab |
| | Foliar Fertilizer | | | 18.1abc |
| | FFI | | | 18.0abc |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

†† Combination of foliar fertilizer, fungicides and insecticide application

**Table 2-7. Foliar application effect on stand counts, grain yield, protein and oil concentrations in Beresford, SD in 2018.**

| Maturity Group | Foliar application treatment | Early Stand | Final Stand | Yield | Protein | Oil |
|---|---|---|---|---|---|---|
| | | (plants ha$^{-1}$) | | (Mg ha$^{-1}$) | (%) | |
| **MG1** | Control | 247200 | 220200 | 5.53b | 35.1 bc† | 18.5 |
| | Fungicide | 252600 | 231800 | 5.62a | 34.7 c | 18.7 |
| | Insecticide | 260000 | 241700 | 5.51b | 34.9 c | 18.4 |
| | Fungicide and Insecticide | 251000 | 238400 | 5.64a | 34.9 c | 18.5 |
| **MG2** | Control | 244400 | 233800 | 5.52c | 35.0 bc | 18.7 |
| | Fungicide | 236200 | 241700 | 5.91a | 35.2 ab | 18.9 |
| | Insecticide | 251800 | 246100 | 5.71b | 35.0 bc | 18.8 |
| | Fungicide and Insecticide | 254300 | 235100 | 5.93a | 35.5 a | 18.7 |
| | *p*<F | | | | | |
| | MG | 0.98 | **0.01** | **0.05** | **<.0001** | **<.0001** |
| | Treatment | 0.92 | 0.33 | **0.003** | 0.10 | **0.01** |
| | MG* Treatment | 0.85 | 0.88 | 0.55 | **0.04** | 0.14 |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level
†† Combination of foliar fertilizer, fungicides and insecticide application

**Table 2-8 Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, and maturity group (MG) main effects and their interactions on leaves, main stem, branches and petioles, pods biomass, and total biomass accumulation at R5, R6, and R7 growth stages in eastern SD in 2018 and 2019.**

| | **Leaves** | **Main Stem** | **Branches + Petioles** | **Pods** | **Total biomass** |
|---|---|---|---|---|---|
| | | | **(*p*<F)** | | |
| | **R5** | | | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Location** | **<.0001** | **<.0001** | **0.001** | **<.0001** | **0.02** |
| **MG** | **0.04** | 0.23 | **<.0001** | **0.008** | **0.01** |
| **Treatment** | 0.98 | 0.11 | 0.56 | 0.17 | 0.53 |
| **Year*Location** | **0.003** | **<.0001** | 0.26 | **<.0001** | **<.0001** |
| **Year*MG** | 0.77 | **0.006** | *0.10* | *0.08* | 0.61 |
| **Location*MG** | **0.02** | **0.02** | 0.27 | *0.05* | 0.39 |
| **Year* Treatment** | 0.70 | 0.30 | 0.25 | 0.20 | 0.78 |
| **Location* Treatment** | 0.21 | 0.64 | 0.20 | 0.44 | 0.36 |
| **MG* Treatment** | 0.6 | 0.42 | 0.33 | 0.61 | 0.71 |
| **Year*Location*MG** | 0.86 | 0.12 | 0.26 | 0.03 | 0.41 |
| **Year*Location* Treatment** | 0.12 | 0.86 | 0.18 | 0.76 | 0.69 |
| **Year*MG* Treatment** | 0.03 | **0.0001** | 0.29 | 0.34 | 0.01 |
| **Location*MG* Treatment** | 0.81 | 0.20 | 0.98 | 0.71 | 0.56 |
| **Year*Location*MG*Treatment** | 0.19 | *0.08* | 0.40 | 0.43 | 0.17 |

**Table 2-8 cont.**

| | Leaves<br><br>**(*p*<F)** | Main Stem | Branches + Petioles | Pods | Total biomass |
|---|---|---|---|---|---|
| | **R6** | | | | |
| **Year** | **<.0001** | **<.0001** | **0.0001** | **<.0001** | **<.0001** |
| **Location** | **<.0001** | **<.0001** | **<.0001** | **0.02** | **0.03** |
| **MG** | ***0.06*** | **0.002** | 0.51 | 0.75 | 0.29 |
| **Treatment** | 0.20 | **0.008** | 0.45 | 0.26 | 0.11 |
| **Year*Location** | **0.006** | **<.0001** | **<.0001** | 0.36 | 0.34 |
| **Year*MG** | **<.0001** | **0.004** | *0.05* | 0.17 | 0.34 |
| **Location*MG** | **<.0001** | **0.002** | **0.003** | *0.08* | 0.11 |
| **Year* Treatment** | 0.52 | 0.13 | 0.10 | 0.20 | 0.43 |
| **Location* Treatment** | **0.04** | *0.05* | 0.28 | 0.14 | *0.06* |
| **MG* Treatment** | 0.59 | 0.25 | 0.95 | 0.70 | 0.83 |
| **Year*Location*MG** | **<.0001** | **<.0001** | **0.04** | 0.91 | **0.03** |
| **Year*Location* Treatment** | 0.58 | 0.60 | 0.88 | 0.69 | 0.70 |
| **Year*MG* Treatment** | 0.62 | 0.33 | 0.70 | 0.57 | 0.81 |
| **Location*MG* Treatment** | 0.75 | 0.70 | 0.49 | 0.69 | 0.76 |
| **Year*Location*MG*Treatment** | 0.24 | 0.23 | 0.12 | 0.66 | 0.30 |

**Table 2-8 cont.**

| | **Leaves (kg ha$^{-1}$)** | **Main Stem** | **Branches + Petioles** | **Seeds / Pod Shells** | **Total biomass** |
|---|---|---|---|---|---|
| | **R7** | | | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001/<.0001** | 0.94 |
| **Location** | **<.0001** | **<.0001** | 0.12 | **0.02/<.0001** | **<.0001** |
| **MG** | **0.007** | *0.06* | 0.99 | **<.0001**/0.18 | 0.93 |
| **Treatment** | **<.0001** | 0.35 | 0.36 | **0.02/**0.11 | 0.15 |
| **Year*Location** | 0.78 | **<.0001** | **<.0001** | 0.62/**<.0001** | **<.0001** |
| **Year*MG** | **0.001** | **0.02** | *0.08* | 0.45/0.66 | **<.0001** |
| **Location*MG** | 0.17 | 0.26 | **0.03** | 0.97/0.26 | 0.97 |
| **Year* Treatment** | 0.47 | 0.55 | 0.98 | 0.12/*0.08* | *0.09* |
| **Location* Treatment** | *0.05* | 0.16 | 0.35 | 0.89/0.72 | 0.37 |
| **MG* Treatment** | 0.69 | 0.68 | 0.92 | 0.15/0.66 | 0.66 |
| **Year*Location*MG** | 0.78 | 0.79 | 0.46 | 0.69/0.24 | 0.63 |
| **Year*Location* Treatment** | 0.30 | 0.40 | 0.85 | 0.72/0.64 | 0.23 |
| **Year*MG* Treatment** | 0.52 | 0.38 | 0.98 | 0.53/0.12 | 0.50 |
| **Location*MG* Treatment** | 0.58 | 0.85 | 0.42 | 0.99/0.99 | 0.77 |
| **Year*Location*MG*Treatment** | 0.60 | 0.68 | 0.65 | 0.94/0.88 | 0.38 |

**Table 2-9. Foliar application, effect on R5, R6, and R7 biomass weights in eastern SD in 2018.**

| | Leaves | Main Stem | Branches + Petioles | Pods; Seeds / Pod Shells | Total biomass |
|---|---|---|---|---|---|
| | | | (kg ha$^{-1}$) | | |
| | | | **R5** | | |
| **UTC** | 2051 | 1606 | 1343 | 2047 | 7047 |
| **Fungicide** | 2020 | 1444 | 1396 | 1852 | 6712 |
| **Insecticide** | 2031 | 1477 | 1435 | 2095 | 7038 |
| **Fungicide and Insecticide** | 2045 | 1528 | 1387 | 2156 | 7116 |
| **Foliar Fertilizer** | 1963 | 1331 | 1352 | 1678 | 6324 |
| **FFI††** | 1969 | 1519 | 1237 | 1770 | 6495 |
| | | | **R6** | | |
| **UTC** | 1657ab† | 1697 | 1254 | 4287 | 8895 |
| **Fungicide** | 1629ab | 1519 | 1180 | 3895 | 8223 |
| **Insecticide** | 1606ab | 1642 | 1213 | 4123 | 8584 |
| **Fungicide and Insecticide** | 1654ab | 1642 | 1241 | 4192 | 8729 |
| **Foliar Fertilizer** | 1482b | 1506 | 1123 | 3876 | 7987 |
| **FFI††** | 1720a | 1797 | 1248 | 4258 | 9023 |
| | | | **R7** | | |
| **UTC** | 206b | 1192 | 694* | 4431b | 6523 |
| **Fungicide** | 348a | 1236 | 800 | 4737ab | 7121 |
| **Insecticide** | 203b | 1333 | 717 | 4737ab | 6990 |
| **Fungicide and Insecticide** | 284ab | 1237 | 715 | 5026a | 7262 |
| **Foliar Fertilizer** | 215b | 1297 | 645 | 4916ab | 7073 |
| **FFI††** | 325a | 1258 | 714 | 4500b | 6797 |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

†† Combination of foliar fertilizer, fungicides and insecticide application

* Branches and petiole fractions at R7 growth stage contained mainly branches, as petioles have fallen

**Table 2-10. Foliar protection maturity group differences, and year by location interaction on leaves, stems, branches, pods, seeds, pod shells, and total biomass.**

| | | | Leaves | Stems | Branches | Pods; Seeds/Pod Shells | Total |
|---|---|---|---|---|---|---|---|
| **R5** | *MG* | | | | | | |
| | MG1 | | 1935b | 1534a | 1306b | 1606a | 6382b |
| | MG2 | | 2030a | 1529b | 1510a | 1599b | 6669a |
| | *Year x Location* | | | | | | |
| | 2018 | Brookings | 2603a | 2484a | 1727a | 1784b | 8598a |
| | 2019 | Brookings | 1597b | 1196b | 1192b | 5057a | 4756b |
| | | SERF | 1562b | 847b | 1040b | 789b | 4239b |
| **R6** | *MG* | | | | | | |
| | MG1 | | 1842a | 1820a | 1435b | 4065a | 9471a |
| | MG2 | | 1797b | 1690b | 1470a | 4012b | 9348b |
| | *Year x Location* | | | | | | |
| | 2018 | Brookings | 2244a | 3020a | 1785a | 5057a | 12601a |
| | 2019 | Brookings | 1530b | 1282b | 1259b | 3287b | 7656b |
| | | SERF | 1549b | 940b | 1138b | 3157b | 7072b |
| **R7** | *MG* | | | | | | |
| | MG1 | | 297b | 1536a | 750b | 3521b/1653b | 8540b |
| | MG2 | | 328a | 1453b | 827a | 3635a/1725a | 8696a |
| | *Year x Location* | | | | | | |
| | 2018 | Brookings | 458a | 2331a | 848a | 2169b/792b | 7813b |
| | 2019 | Brookings | 126c | 1021b | 632b | 2945b/2311a | 7587b |
| | | SERF | 317b | 962b | 652b | 4354a/1760a | 8024a |

**Table 2-11. Foliar application treatment effect on the relative biomass partitioning at the different growth stages across maturity groups and site-years in 2018 and 2019.**

| | Leaves | Main Stem | Branches and Petioles | Pods; Seeds/Pod Shells | Fallen Leaves |
|---|---|---|---|---|---|
| | | | % | | |
| | | | **R5** | | |
| **UTC** | 0.36 | 0.24 | 0.19 | 0.14 | N/A |
| **Fungicide** | 0.34 | 0.21 | 0.24 | 0.14 | N/A |
| **Insecticide** | 0.33 | 0.24 | 0.22 | 0.14 | N/A |
| **Fungicide and Insecticide** | 0.33 | 0.23 | 0.23 | 0.14 | N/A |
| **Foliar Fertilizer** | 0.32 | 0.21 | 0.22 | 0.17 | N/A |
| **FFI††** | 0.31 | 0.23 | 0.19 | 0.18 | N/A |
| | | | **R6** | | |
| **UTC** | 0.19 | 0.18 | 0.14 | 0.47 | 0.03ab |
| **Fungicide** | 0.21 | 0.17 | 0.14 | 0.46 | 0.02ab |
| **Insecticide** | 0.18 | 0.19 | 0.13 | 0.47 | 0.03ab |
| **Fungicide and Insecticide** | 0.18 | 0.18 | 0.15 | 0.47 | 0.02b |
| **Foliar Fertilizer** | 0.18 | 0.18 | 0.14 | 0.46 | 0.04a |
| **FFI††** | 0.20 | 0.19 | 0.14 | 0.45 | 0.03ab |
| | | | **R7** | | |
| **UTC** | 0.02 | 0.13 | 0.07 | 0.44/0.20 | 0.13 |
| **Fungicide** | 0.04 | 0.14 | 0.08 | 0.44/0.21 | 0.10 |
| **Insecticide** | 0.02 | 0.15 | 0.08 | 0.46/0.21 | 0.09 |
| **Fungicide and Insecticide** | 0.02 | 0.15 | 0.07 | 0.45/0.19 | 0.11 |
| **Foliar Fertilizer** | 0.04 | 0.12 | 0.06 | 0.45/0.23 | 0.12 |
| **FFI††** | 0.03 | 0.15 | 0.06 | 0.45/0.22 | 0.11 |

†† Combination of Fungicide, Insecticide, and Foliar Fertilizer

**Table 2-12 Analysis of Variance (ANOVA) significance levels of pest and pathogen on foliar protection application, year, location, and maturity group (MG), and timing of rating (SBS, Septoria Brown Spot; SDS, Sudden Death Syndrome) in eastern SD in 2018 and 2019.**

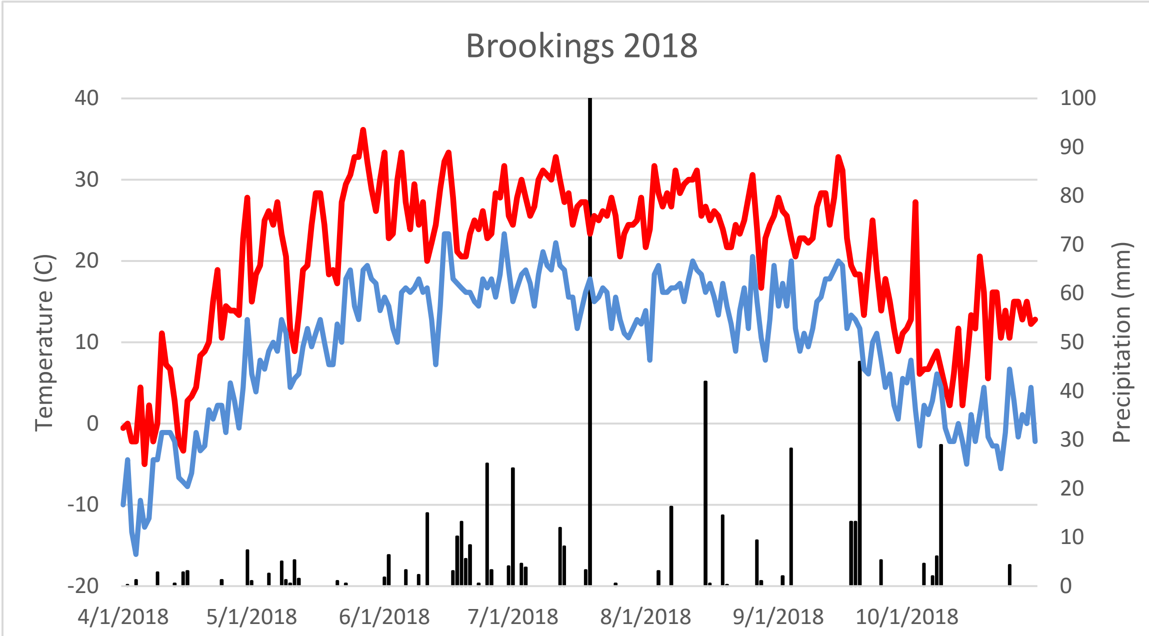
| | Pest | SBS | SDS |
|---|---|---|---|
| **Year** | **<.0001** | **<.0001** | **0.01** |
| **Location** | **<.0001** | 0.35 | **0.01** |
| **Treatment** | **<.0001** | 0.68 | 0.74 |
| **MG** | **<.0001** | 0.93 | 0.37 |
| **Timing** | **<.0001** | **<.0001** | **0.002** |
| **Year*Location** | **<.0001** | *0.06* | **0.02** |
| **Year*Treatment** | **<.0001** | 0.92 | 0.48 |
| **Location*Treatment** | **0.01** | 0.65 | 0.38 |
| **Year*MG** | **<.0001** | 0.63 | 0.35 |
| **Location*MG** | 0.30 | 0.48 | 0.36 |
| **Treatment*MG** | **<.0001** | 0.74 | 0.99 |
| **Year*Timing** | **<.0001** | **<.0001** | **0.002** |
| **Location*Timing** | **<.0001** | **0.02** | **0.002** |
| **Treatment*Timing** | **<.0001** | 0.94 | 0.85 |
| **MG*Timing** | **0.03** | 0.99 | 0.44 |
| **Year*Location*Treatment** | **<.0001** | 0.64 | 0.49 |
| **Year*Location*MG** | 0.33 | 0.69 | 0.38 |
| **Year*Treatment*MG** | **0.002** | 0.52 | 0.99 |
| **Location*Treatment*MG** | *0.07* | 0.27 | 0.99 |
| **Year*Location*Timing** | **<.0001** | 0.15 | 0.004 |
| **Year*Treatment*Timing** | **<.0001** | 0.94 | 0.53 |
| **Location*Treatment*Timing** | 0.74 | 0.80 | 0.38 |
| **Year*MG*Timing** | **0.02** | 0.80 | 0.42 |
| **Location*MG*Timing** | 0.77 | 0.62 | 0.43 |
| **Treatment*MG*Timing** | 0.23 | 0.89 | 1.00 |
| **Year*Location*Treatment*MG** | 0.15 | 0.82 | 0.99 |
| **Year*Location*Treatment*Timing** | **0.008** | 0.77 | 0.54 |
| **Year*Location*MG*Timing** | 0.83 | 0.90 | 0.46 |
| **Year*Treatment*MG*Timing** | 0.20 | 0.46 | 0.99 |
| **Location*Treatment*MG*Timing** | 0.68 | 0.52 | 0.99 |
| **Year*Location*Treatment*Timing** | 0.89 | 0.89 | 0.99 |

**Table 2-13. Pest and pathogen disease (SBS Septoria Brown Spot, SDS Sudden Death Syndrome) ratings between Treatments, Timing of ratings, MG in Brookings and Beresford, SD in 2018 and 2019.**

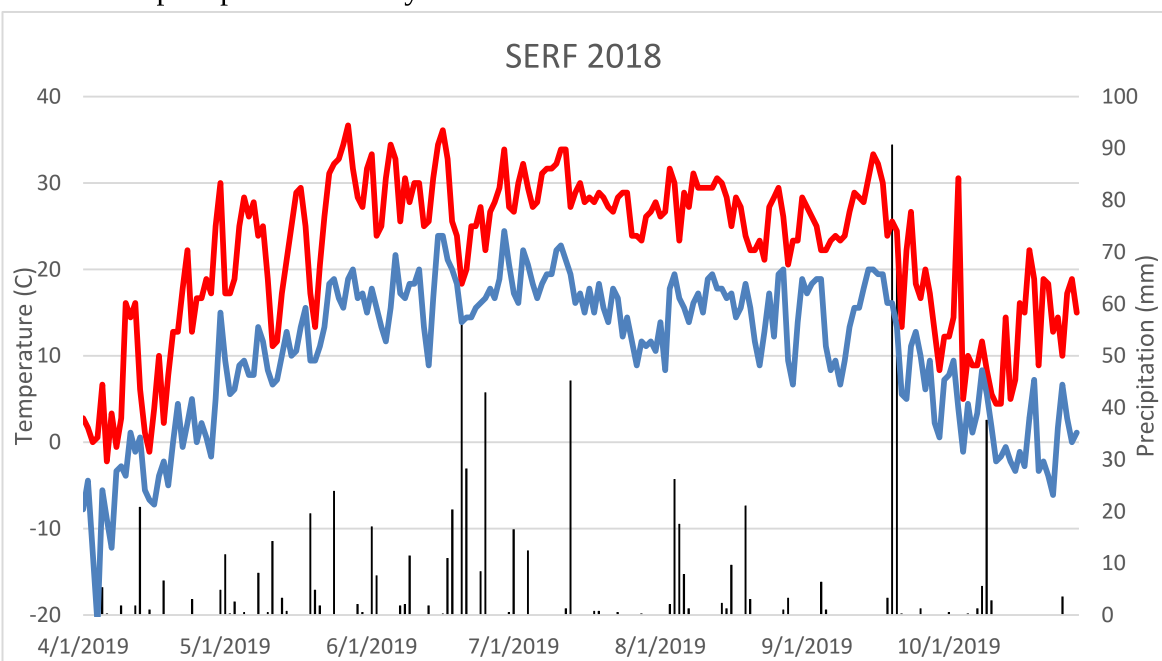
| | Pest | SBS | SDS |
|---|---|---|---|
| ***Location*** | | | |
| **Brookings** | 2.98a | 4.51 | 0.89 |
| **SERF** | 2.35b | 4.13 | N/A |
| ***Timing*** | | | |
| **Pre** | 1.05c | 0.70b | N/A |
| **14 DAA** | 2.98b | 1.83b | N/A |
| **28 DAA** | 3.97a | 10.43a | 1.33 |
| ***Timing* Treatments*** | | | |
| **0 DAA Control** | 1.13de | 0.81b | 0.41c |
| **0 DAA Fungicide** | 1.03e | 0.69b | 0.34c |
| **0 DAA Insecticide** | 1.03e | 0.63b | 0.31c |
| **0 DAA Fungicide and Insecticide** | 1.06e | 0.69b | 0.34c |
| **0 DAA Foliar Fertilizer** | 1.13de | 0.69b | 0.34c |
| **0 DAA FFI**†† | 0.91e | 0.69b | 0.34c |
| **14 DAA Control** | 4.46ab | 2.22b | 2.06ab |
| **14 DAA Fungicide** | 3.41b | 1.84b | 1.93ab |
| **14 DAA Insecticide** | 1.81cde | 2.06b | 2.13ab |
| **14 DAA Fungicide and Insecticide** | 1.91cde | 1.63b | 1.75b |
| **14 DAA Foliar Fertilizer** | 4.34ab | 1.84b | 1.94ab |
| **14 DAA FFI**†† | 1.97cde | 1.41b | 1.81ab |
| **28 DAA Control** | 5.69a | 11.13a | 2.4ab |
| **28 DAA Fungicide** | 4.19ab | 9.09a | 2.22ab |
| **28 DAA Insecticide** | 2.63cd | 11.38a | 2.66a |
| **28 DAA Fungicide and Insecticide** | 2.94bc | 9.13a | 1.91ab |
| **28 DAA Foliar Fertilizer** | 5.41a | 12.44a | 2.53a |
| **28 DAA FFI**†† | 3.00bc | 9.43a | 2.03ab |
| ***Year*location*** | | | |
| **2018 Brookings** | 0.84c | 5.90a | 1.77a |
| **2019 Brookings** | 5.12a | 3.12b | 0b |
| **2019 SERF** | 3.20b | 1.99b | 0b |

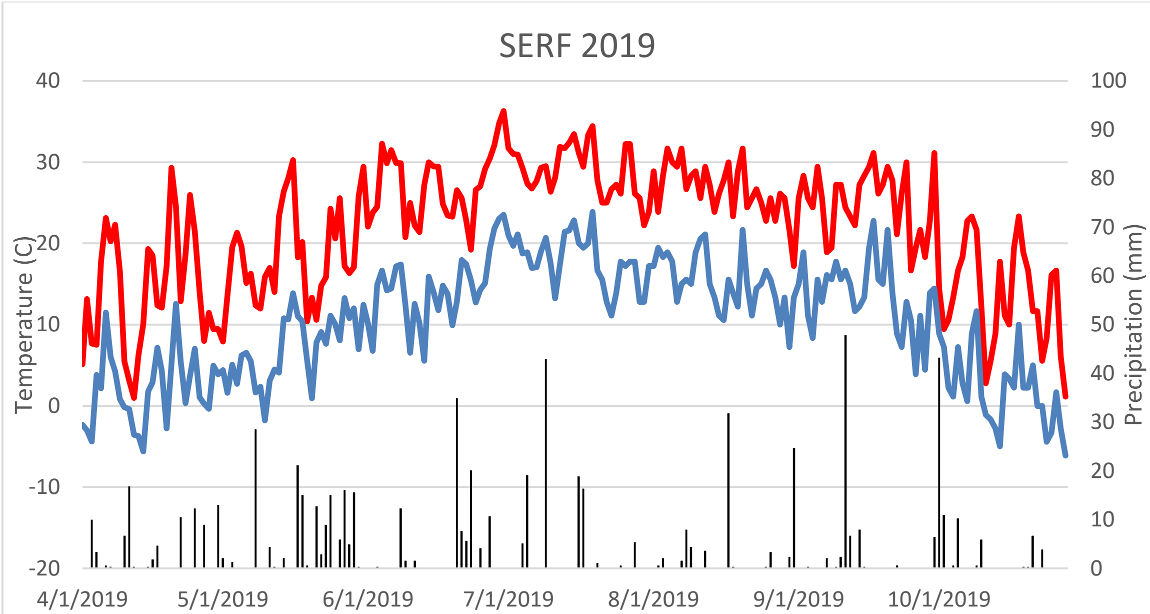
†† Combination of Fungicide, Insecticide, and Foliar Fertilizer

**Figure 2-1. Daily minimum and maximum air temperature and precipitation amount near Brookings and Beresford, SD in 2018 and 2019.**




* 159.3mm precipitation on July 19th




SERF 2019
Temperature (C)
Precipitation (mm)
40
30
20
10
0
-10
-20
100
90
80
70
60
50
40
30
20
10
0
4/1/2019
5/1/2019
6/1/2019
7/1/2019
8/1/2019
9/1/2019
10/1/2019

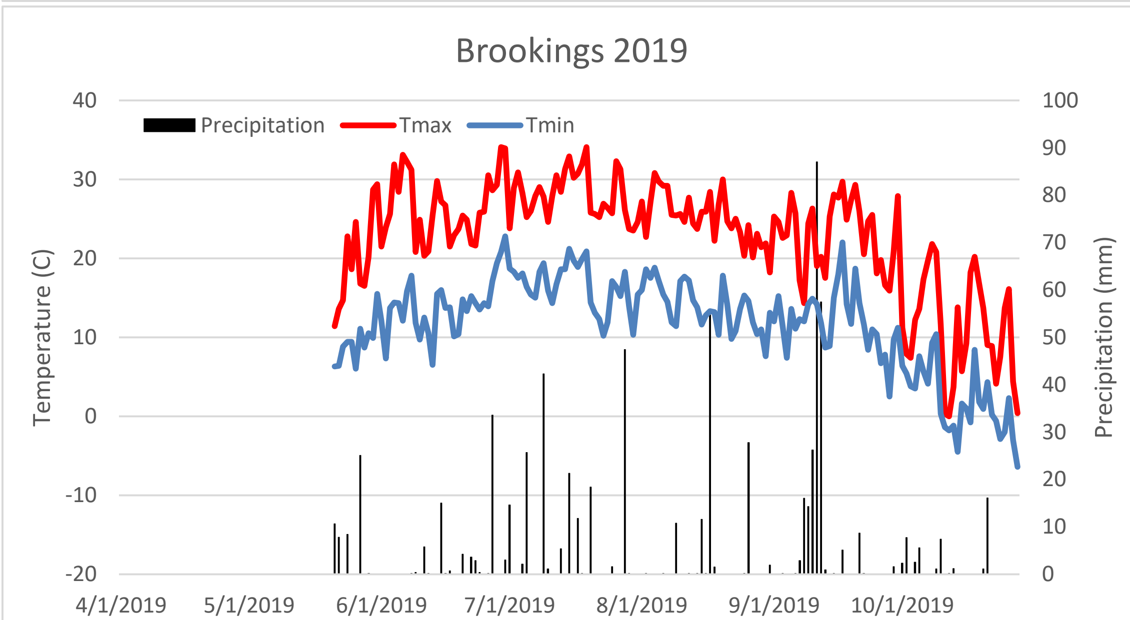
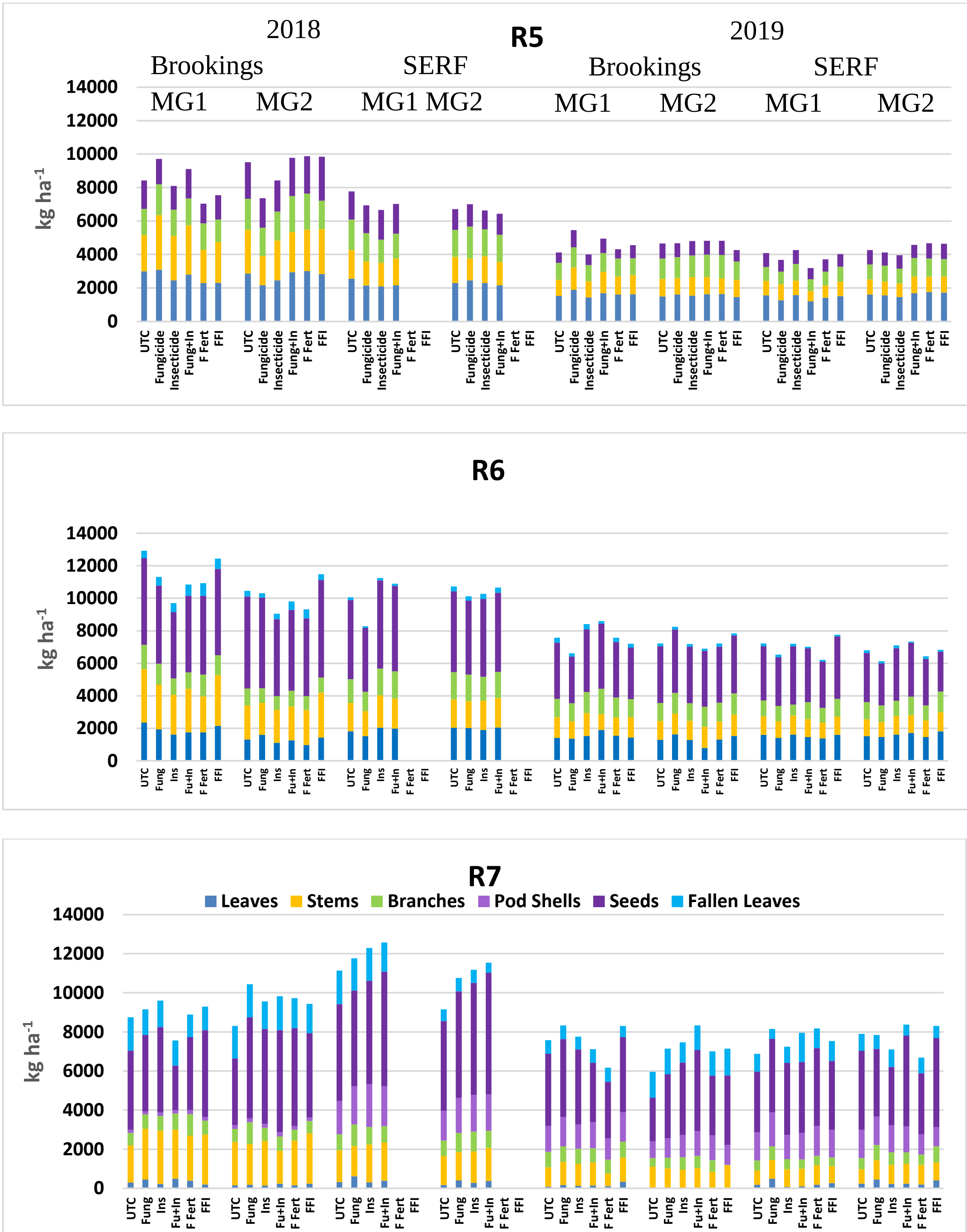

Brookings 2019
Precipitation
Tmax
Tmin
Temperature (C)
Precipitation (mm)
40
30
20
10
0
-10
-20
100
90
80
70
60
50
40
30
20
10
0
4/1/2019
5/1/2019
6/1/2019
7/1/2019
8/1/2019
9/1/2019
10/1/2019

**Figure 2-2. Foliar application, R5, R6, R7 biomass weights in eastern SD in 2018 and 2019.**




†† Combination of foliar fertilizer, fungicides and insecticide application

# CHAPTER 3
# NITROGEN AND SULFUR EFFECT ON SOYBEAN SEED COMPOSITION

## 3.1 INTRODUCTION

The amount of fixed N used by a plant is often largely dependent on N availability in the soil, with the plants utilizing available soil N prior to N gained from N fixation (Salvagiotti et al., 2009a). Soybeans fix about from 25 to 75 percent of their N according Salvagiotti et al. (2008), and from approximately two to three weeks after planting to pod fill (Flynn et al., 2015). Adding N fertilizer to the soybeans later during the growing season can aid with the needs of the crop when it is unable to fix enough for itself. Protein synthesis is the process of forming amino acids into proteins. Nitrogen is one of the major components of these amino acids that are the building blocks of proteins (Galili and Galili., 2008). Nitrogen and S applications during the growing season could help increase grain quality, therefore, applying N can cause the soybeans to be less stressed in the reproductive stages which may aid in the achievement of a higher seed protein percentage. Late application of N around R3 (beginning seed) tended to increase the percentage of protein in the seed, but yield was not affected (Wesley et al. 1997). In a study conducted using several cultivars with different maturity lengths, remobilization of N from the plant to the seed ranged from 30 to almost 100 percent, with more N being remobilized in southern regions due to a longer growing season (Zeiher et al., 1982). While there has not been a considerable amount of research done that focuses solely on the composition of soybeans; a study by Schmitt et al. (2001) showed a positive response in grain protein concentration when late season (R2-R5) N is applied. Available N in the soil is crucial during periods of peak N demand, such as grain fill period. "In general, soybean yield correlates closely with total N in the plant because of the large amount of N accumulation in the seed" (Kuwahara 1986). Modern cultivars have a larger amount of biomass and N accumulation than older cultivars (Schmitt et al., 2001). Modern cultivars' nutrient uptake occurs longer during the grain fill period than older varieties (Kovács and Casteel, unpublished). While N composition of the soybean is important, there are additional S requirements needed for the soybean plant to reach its full potential. Sulfur is a constituent of key enzymes in N metabolism (Campbell, 1999; Friedrich and Schrader, 1978) and in legumes is associated with the enzymes that are responsible for biological N fixation (Benton and Peters, 2004). Sulfur is key in plant metabolism and part of essential amino acids in seed storage proteins (Losak et al., 2010; Sexton et al., 1998; Takahashi et al., 2011). Pulse applied $^{35}S$ research documented that more S translocated to the seed when taken up later, during seed development (Naeve et al., 2005). Salvagiotti et al. (2009b) showed a rise in N uptake in response to S addition that increased biomass production, via a larger radiation capture, which then increased soybean yield (Salvagiotti et al., 2009b).

Historically, S fertilization has not been a concern for most of the farmers due to enough sulfate ($SO_4^{2-}$) deposition from the environment for the crop to flourish. Typically, low organic matter and coarse textured soils were considered to be at risk for S deficiency (Brady and Weil, 1999). There has been a reduction in sulfur deposition because of the increase in control of S released from industry and the use of low S grade fertilizers (Scherer, 2001). With recent pollution reductions, research has found that applications of sulfur are needed in other soil conditions as well (Brady and Weil, 1999).

In addition, with the increased grain yield, S uptake also increased in modern soybean varieties (Kovács and Casteel, unpublished).

Nitrogen and S should be given in balanced doses to obtain optimum yield (Jamal et al., 2005). “In seeds of Ivy (*Hedera helix* L.), Obeso et al. (2012) showed an allometric relationship between S concentration and seed mass. Isometric variations between seed mass and N concentration in Ivy seeds suggest allometric relationships between N and S. In soybeans Salvagiotti et al., (2012) found that for a given amount of N accumulated in seeds, S deficient soybeans tended to accumulate 12% less S in seeds than soybeans with no S limitations.

Protein quality can change: “If increased demand for S-containing amino acids within the seed did create a source limitation in S uptake or reduction, then either protein yield would drop to maintain quality, or the seed would synthesize greater amounts of poorer quality protein than expected” (Sexton et al., 1998). Not only is increasing protein levels important, but maintaining the quality of the protein is important when considering fertilizing soybeans.

The objectives of this study were to 1) determine the effects of N and S application rate and timing on grain yield and protein levels in soybean seeds in different maturity group varieties, and 2) determine the effect of planting dates.

## 3.2 MATERIAL AND METHODS

### 3.2.1 Site description and experimental design

This study was conducted at two of the South Dakota State University Research Farms; near Brookings (44.3114° N, 96.7984° W), and at the Southeast Research Farm (SERF; 43.0805° N, 96.7737° W) near Beresford, SD. The soil types were Divide (fine-loamy over sandy, mixed, superactive, frigid Aeric Calciaquolls), and Egan-Wentworth complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at Brookings and Egan-Clarno-Tetonka complex, which is a fine-loamy/fine-silty, mixed, superactive, mesic Typic Haplustolls; Egan-Clarno-Trent complex, which is a fine-silty, mixed, superactive, mesic Udic Haplustolls at SERF in 2018 and 2019, repsectively.

Two soybean varieties, AG11X8, AG24X7 (maturity group 1.1 and 2.4, respectively, AsGrow Seed Co LLC, St. Kalamazoo, MI) were included in this research. Different N S fertilizer application timings were compared in each variety: untreated control, application at planting, application at V4 (four trifoliate), at R3 (beginning pod), or at V4 and R3 growth stages. Assigned plots received 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ at each application timing. Sulfur was applied as ammonium-sulfate (AMS; 21-0-0-24S) and urea (46-0-0) was used to supplement the remainder N at pre-plant and V4 growth stage. These applications were broadcast applied by hand. The R3 applications were done with a one nozzle wand next to the base of the plant with AMS and Urea-ammonium nitrate (UAN 28-0-0) at a total of 140 L $ha^{-1}$ application rate. At the Brookings sites an additional, delayed, second planting date was also included. Treatments were arranged in a randomized complete block design at SERF and with split-plot arrangement in Brookings, with 4 replications at each location. At the Brookings site the main plot was the planting date and sub plots were the fertilizer application timing and soybean maturity group combinations.

Corn (*Zea mays L.*) was the preceding crop at both locations in 2019 and the Brookings site in 2018. These sites utilized conventional tillage practices and prior to planting fields were field cultivated, while at Beresford in 2018 soybean were planted in no-till ground following oats (*Avena sativa* L.). In 2019, soybeans at the Beresford site required replanting due to very poor plant stand caused by wet weather conditions during planting and emergence window.  All plots were maintained weed free. Soybean varieties were planted at 346,000 seeds $ha^{-1}$ in 76-cm rows; the planting dates for each sites are presented in Table 3-1.  Plot sizes were 3m x 18.3m in Brookings in 2018 and 4.5m x 13.7m at SERF both years and Brookings in 2019.

3.2.2 Data collection

Before planting, soil samples were taken from each replication (15 cores of 2 cm diameter in each replication) and separated to 0-15cm, 15-60cm; soil samples were air dried with forced air then ground to pass through a 2 mm sieve, and sent off to a certified commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient concentrations, soil pH and organic matter (OM). The laboratory used the 1:1 soil-water slurry method for soil pH, the loss of ignition method for OM, the cadmium reduction method for $NO_3^-$-N and the monocalcium phosphate method for $SO_4^{2-}$—S concentration determination (AgSource Laboratories, 2020).

Pre-plant fertilizer application was done on the same days as planting. The in-season fertilizer application times presented in Table 3-1.

After emergence (around V3 growth stage) and at physiological maturity, plant population was estimated through stand counts at four locations within each plot. The number of plants were counted on one meter length of a non-border row and plant population were calculated from it.

Most recent matured trifoliate leaf (MRML) samples were taken from 25 plants in each plot in Brookings and at the SERF in July and August in 2018 (Table 3-1). Samples were dried then ground to pass through a 1 mm sieve, and analyzed by a certified commercial laboratory (AgSource Laboratories, Lincoln, NE) to determine nutrient concentrations. Nitrogen concentration was determined by the Kjeldahl method, P, K, Mg, Ca, S, Zn, Mn, Cu, Fe, B, Al concentrations were determined by the inductively coupled argon plasma spectrometry method (AOAC International, 2000; AgSource Laboratories, 2020). At R5 (beginning seed), R6 (full seed), and R7 (beginning maturity) growth stages biomass samples were collected from a 0.5m section of a non-border row. Sampling times are presented in Table 3-1. The R5 and R6 samples were partitioned into leaves, stems, branches (including petioles), pods, and fallen leaves. The R7 samples were partitioned into leaves, stems, branches (including petioles), pod shells, seeds, and fallen leaves. Fallen leaves were any leaves or petioles that fell in the area biomass was collected from 0.38 m each side of the sampled row. All samples were dried at 60 degrees Celsius until constant weight then weighed.

Grain harvest was done in late October (Table 3-1). The middle two rows being harvested with a Massey Ferguson 8XP plot combine. Grain weight, grain moisture and test weight readings were recorded. Plot lengths were measured to determine grain yield. Grain protein and oil concentrations were determined with InfraTec Nova (FOSS Analytics, Hillerød, Denmark) instrument. Seed mass (200-seed weight) were measured by weighing grain samples collected from the harvested plots. Grain yield, grain protein and oil, and the 200 seed weight data was adjusted to 130 g $kg^{-1}$ moisture content.

### 3.2.3 Statistical analysis

Results were then analyzed in R Studio and interpreted using ANOVA and Fishers-LSD at 0.05 significance level. The two site years from SERF, and the first planting dates at the Brookings site were analyzed together for treatment differences. Further ANOVA analysis were carried out for the Brookings sties, where both planting dates from both year have been included in the analysis to compare the effect of planting dates. All treatment factors (fertilizer application timing, MG, year, location, and planting date) were considered fixed factors.

## 3.3 RESULTS

### 3.3.1 Weather

These experiments located side by side with the experiments presented in Chapter 2. Detailed information about weather conditions (daily and monthly minimum and maximum air temperature, and daily and monthly total precipitation) were described in Chapter 2.3.1.

### 3.3.2 Field Characterization

Preplant soil sample tests indicated soil pH levels between 6.1 and 6.9, and between 6.2 and 7.5, while OM levels ranged between 3.0% and 4.8%, and between 2.6% and 3.6%; in the 0-15cm and 15-60cm soil depths, respectively, among the experimental sites (Table 3-2). Soil nutrient levels ranged between 3.3ppm and 19.3ppm and between 2.8ppm and 8.0 ppm for $NO_3^-$-N, and nutrient levels were between 3.0ppm and 12.3ppm and between 2.5ppm and 8.5ppm for $SO_4^{2-}$—S in the 0-15cm and 15-60cm soil layers, respectively, among the sites (Table 3-2).

### 3.3.3 Stand Counts

When comparing SERF and Brookings, neither early season nor final plant stands differed among the fertilizer application treatments (Table 3-3). Early season plant stand differed only between the locations and the MGs (Table 3-3). Plant stands were approximately 12,400 plants ha$^{-1}$ larger in MG2 compared to MG1 when plots were averaged across locations, growing seasons and fertilizer treatments. Early season plant stands were approximately 24,700 plants ha$^{-1}$ higher at the Brookings site relative to the SERF site averaged across the fertilizer treatments, MGs, and growing season (Table 3-4). Final plant stands varied between the MGs and by the year by location interaction (Table 3-3). Maturity group 1 variety had nearly 10,000 plants ha$^{-1}$ larger final plant stand than MG2 varieties across fertilizer application timings, growing seasons and locations, while final plant stand was 30,000 plants ha$^{-1}$ larger in 2019 than in 2018 averaged across fertilizer application timings, MGs, and locations (Table 3-4).

When comparing the two planting dates at Brookings, larger plant population were established with the second planting date compared to the first planting date (Tables 3-6 and 3-7); plant population differed by 30,000 plant ha$^{-1}$ at the early stand counts and by 10,000 plants ha$^{-1}$ for the harvest stand counts averaged across the fertilizer applications, MGs and growing seasons. Plant stands in 2019 were higher (by about 10,000 plants ha$^{-1}$ for the early season estimation and by 60,000 plants ha$^{-1}$ in the harvest stand counts) when averaged across fertilizer treatments, MGs, and planting dates (Table 3-6 and 3-7).

### 3.3.4 Grain Yield, Protein and Oil concentrations

When comparing across locations, as shown in Table 3-3, statistical analysis showed that there were no significant differences in grain yield among nutrient application timings across locations. However, the year by location interaction (Table 3-3) revealed that grain yield was nearly 0.9 Mg $ha^{-1}$ higher at SERF in 2018 compared to the Brookings site averaged across fertilizer treatments and MGs (Table 3-5). While numerically 0.35 Mg $ha^{-1}$ higher but statistically not different yield was achieved in the same comparison in 2019 (Table 3-5). When we look at the location by MG interaction (Table 3-3), MGs did not differ within a location averaged across the fertilizer application timings and growing seasons, but numerically MG2 varieties had higher yield at Brookings, while MG1 varieties achieved numerically higher yield at the SERF site (Table 3-5). The SERF site in 2018 had the highest grain yield and grain oil concentration (Table 3-4). For grain protein, the Brookings site had nearly 1% higher grain protein concentration compared to the SERF site in 2018, while in 2019 grain protein was numerically higher at SERF (by about 0.45%) but not statistically different between the sites (Table 3-5). Grain protein concentration ranged from 33.6-36.2 percent, in both locations (Tables 3-5. The year by location by MG interaction for grain oil concentration (Table 3-3) indicated that grain oil concentration were higher at the SERF site in 2018 compared to the Brookings location and MGs did not differ, while in 2019 MG2 varieties had higher grain oil concentration by about 0.5% in both locations (Table 3-5). As shown on Table 3-4, grain oil concentration were higher by about 0.15% in V4+R3 fertilizer treatment than the pre-plant application, but neither of these treatments were different from the untreated check and other fertilizer application timings.

When comparing the planting dates at the Brookings site, fertilizer application timing treatments were also not different for grain yield, protein, or oil concentrations (Table 3-6). Only when looked at on the 0.1 significance level is fertilizer application timing impacted grain protein concentration (Table 3-6). The year by planting date interaction indicated a 0.55 Mg $ha^{-1}$ yield advantage for early planting in 2018 while there were no differences among the planting dates in 2019 averaged across the fertilizer timing and MGs (Tables 3-6 and 3-8). The year by fertilizer application interaction showed no yield differences between the growing seasons when N and S fertilizer was applied, but the non-treated control had 0.45 Mg $ha^{-1}$ lower yield in 2019 averaged across the MGs and planting dates (Table 3-8). In 2019, the first planting date yielded higher grain oil concentration than any other planting dates in the two years. Grain yield and protein concentrations were the highest in 2018 with the first planting date (Table 3-8). Grain protein concentration ranged from 33.8-36.0 percent, over both planting dates (Tables 3-8). In 2018, grain protein concentration was higher in the first planting date; while in 2019, it was higher in the second planting date (Table 3-8).

### 3.3.5 Biomass

When comparing the two locations, biomass partitions did not differ due to fertilizer application timing treatments at either growth stages across the four site-years (Table 3-9 and 3-10). Branches and petioles were collected together and could be why we see a dramatic decrease (1722 to 817kg $ha^{-1}$) from R6 to R7 biomass weights (Table 3-10). While Brookings was similar to SERF in leaves and pods biomass at R5 and R6; there was significantly higher (~500 kg $ha^{-1}$ in R5 ~1000 kg $ha^{-1}$ R6) stems and branches biomass in the Brookings site than at the SERF site (Figure 3-1). Total biomass in 2019 was lower (more than 1000 kg $ha^{-1}$) than in 2018. The biomass partitioning was also calculated as the relative proportion of the biomass accumulation for each treatment (Table 3-11) to see if there were difference between the biomass allocation relative to the total biomass accumulation. Results indicated that even though total biomass accumulation may had differed, the proportion of biomass allocation within the plant was not significant (Table 3-11).

When comparing the planting dates, biomass accumulation was influenced by the year by planting date interaction (Table 3-12). At the R6 growth stage, in 2018 the second planting date accumulated less (~1000 kg $ha^{-1}$) total biomass than the first planting date (Table 3-13, Figure 3-2). Overall, in 2019 there were lower biomass accumulation then in 2018. The biomass partitioning was also calculated as the relative proportion of the biomass accumulation for each treatment (Table 3-14).There were no noticeable differences between the planting dates on biomass partitioning in this study (data not shown).

### 3.3.6 Most Recent Matured Leaf

Results from the MRML samples in 2018 when comparing the fertilizer application timings showed differences between N and S concentrations (at the 0.10 significance level (Table 3-15). Sulfur tissue concentration was also higher at the SERF site by 0.03% (Table 3-15). Nitrogen concentration in the control treatments were lower than the fertilized plots averaged across MGs, locations, and growing seasons (Table 3-15).

When comparing the planting dates at Brookings, planting date influenced the leaf K nutrient concentration (p=0.06; Table 3-16). Only the S nutrient concentrations varied due to fertilizer application timings, which was also significant at the 0.10 significance levels averaged across planting dates, and MGs (Table 3-17). Nitrogen concentration in the unfertilized treatments (control and R3) were numerically lower than the fertilized plots (Table 3-16).

## 3.4 DISCUSSION

### 3.4.1 Field Characteristics

The pre-season soil tests taken are shown in Table 3-2 to help show there were adequate pre-season nutrients in the field which can be the reason why we did not see a large increase in yield from applying nitrogen and sulfur throughout the growing season. If fertilizing with N and S would improve grain quality to the field that were not lacking proper amounts in the soil, then we could assume N and S increase protein levels.

### 3.4.2 Grain yield, and quality

Location effect on yield is related to environmental factors such as the higher early season precipitation and warmer temperatures (Rowntree et al., 2013a) during the 2018 growing season at the SERF location. Brookings received a one-time large amount of rain (nearly 160 mm on July 19), which caused substantial lodging in those plots. Lower grain yield with delayed planting is well documented (De Bruin and Pedersen, 2008), which is caused by shorter time for the plant to grow before it starts the reproductive growth and reaches maturity. Later planted soybeans also produce a lower number of nodes per plant (Specht, 2007). The nodes are where the soybeans produce its pods so with fewer pods will cause lower yield. Delayed planting of long or longer maturity group varieties increase the chances that the soybeans will not fully mature before the first frost event. In 2018, the first frost occurred before the MG2 variety fully matured (October 4, Figure 1-2) causing the lower grain yields and protein levels for those treatments compared to the late planted shorter variety or the treatments planted at the optimal planting time (first planting date).  The early frost disrupts photosynthesis, and grain filling which led these MG2 variety treatments with lower yields and lower protein concentrations even though later planting is said to have lower yield and higher protein concentration (Jaureguy et al., 2013, Rowntree et al, 2013a).

Contrary to some research (Kane et al., 1997; Robison et al., 2009), seed protein concentration did not increase within the maturity group when planted in June rather than in May (Pedersen and Lauer, 2003). Grain oil concentration on the other hand, had a larger concentration in MG2 variety than in the MG1 variety regardless of the fertilizer application timing. The pre-plant fertilizer application lowered grain oil concentration compared to the control treatments.

In Brookings 2019, lower levels of residual $NO_3^-$-N and $SO_4^{2-}$-S were found in the soil at the pre-plant sampling time (Table 3-2) which would also considered to be close to low levels according to the current recommendation guidance (Clark, 2020). This could attribute to the lower amounts of protein in these treatments, even though they were planted later which is supposed to increase grain protein concentration (Jaureguy et al., 2013, Rowntree et al., 2013a).

While plant stand was not a problem in this study, making sure that your planter is properly calibrated will help ensure that the field is planted uniformly and that it will not affect yield. Yield and grain quality can also be impacted by maturity groups and planting dates.

Selecting the right maturity group or variety can help increase the productivity of the soybeans in the field. Selecting the right maturity group also depends on when you are planting. Planting dates can affect soybean germination. Soybeans need a soil temperature of at least 10 °C (Hall et al., 2012) in order for them to germinate meaning that if they are planted too early they will have problems with germination which will lead to an uneven stand. This was also observed through higher early season plant stand when planting was delayed and temperatures were also warmer (Table 3-7). The longer the planting is delayed, the shorter the maturity group for the area should be planted. Planting dates that crop insurance covers in South Dakota range from April 25$^{th}$ to June 10$^{th}$, and planting before or after this time frame it is not recommended. In 2019, the second planting date at Brookings was planted three days after what would been covered by crop insurance. While all of the others were within the dates of crop insurance. As in the Assefa et al. (2019) article, the two locations were located between the 40 and 45 degree latitude, which showed a decline in oil concentration and protein concentrations as planting date was delayed. Maturity group selection is also easy to control to help insure the best results from the field.

### 3.4.3 Nutrients and Plant Biomass

Some of the biomass weights differences occurred because timing of sample collection may not have happened at the exact plant developmental stages desired due to the large amount of plots that needed to be collected and partitioned during the grain filling period. Both maturity groups' samples at the R5 growth stage were collected at the same time while in later stages they were collected separately when the different MGs reached the target growth stage. The same sampling time can explain biomass accumulation differences between MGs at the R5 growth stage. There were more distinct time separation between locations and maturity groups as the grain filling period progressed. Node number could have also affected the pods per plant from the two years. Planting occurred earlier in the season in 2018 and planting date one had a longer time before the summer solstice, this leads to a longer amount of time that the plant can grow and produce nodes. Higher numbers of nodes likely lead to a larger number of pods per plant. This could be why there was higher grain yield in 2018.

Some of the treatments that are higher in Figure 2-1 and 2-2 are due to a lower number of plants collected (1 or 2 plants). When there were less plants collected, the soybean plants had more branches. These plants also tended to have more leaves due to the number of branches.

Due to all of the rain received during the growing season, the nitrogen that was plant available may have leached rather than being utilized by the plant. Nitrogen is not normally applied to soybeans due to the fact that soybeans are legumes. This means that they produce their own N through nodules. Abendroth et al. (2006) and Tein et al. (2002) found that soybeans normally acquire between 60 and 89 percent of their total N uptake from N-fixation. Early season N application can interfere with nodule formation on the plant when large amounts of N are applied due to adequate amounts of available N in the soil. Soybeans use the available soil N before initiating nodule formation and N fixation.

This could lead to lower amounts of N fixation, especially later in the growing season, when early season N (from fertilizer application or from mineralization) has been utilized by the plant or leached away.

Sulfur in the past was not a problem due to the amount of S deposition from the atmosphere. Now with emission restrictions, S deficiencies and response to S application have become more prevalent (Scherer, 2001). Sulfur levels are also not typically a problem in South Dakota unless weather is cool and wet, or when production is on coarse textured soils or on low organic matter soil (Gelderman et al., 2000). Soil pre-plant S levels were sufficient according to current South Dakota recommendation guidance (Clark, 2020) which helps to explain why we did not see grain yield response when applying sulfur during the season similarly to the observations of Flavio et al. (2007).

Future research will have to be conducted too further investigate if different rates or different types of N, and S, and their ratios will increase the amount of grain protein found in the soybean seed.

## 3.5 CONCLUSION

When looking at both locations and both planting dates in Brookings, there might have been more significant differences in protein and yield levels had the fields that were in the study were deficient in N and S. While N and S application timings did not seem to affect fields with adequate amounts of nutrients in the soil it could potentially increase if the field was known to have these deficiencies. In this study application of supplemental N and S fertilizers did not improve grain yield or protein concentrations on fields that were already sufficient in these nutrients. While Brookings in 2019 had the lowest amount of N and S in the pre-season soil samples, applications of N and S did not affect grain yield or quality. In the future, looking for nodules on the roots and taking soil samples after harvest would be a way to find out how these applications influenced the soybean.

**Table 3-1.Experimental design, used varieties and other crop production parameters, along with planting, treatment application, biomass and machine harvest dates.**

| | **2018** | | **2019** | |
|---|---|---|---|---|
| ***Field activities*** | **Brookings** | **SERF** | **Brookings** | **SERF** |
| **Varieties** | AG11X8 and AG24X7 | | | |
| **Row spacing** | 0.76m | | | |
| **Replications** | 4 | | | |
| **Seeding Rate** | 346,000 viable seeds ha$^{-1}$ | | | |
| **Plot dimensions** | 3m x 18.3m | 4.5m x 13.7m | | |
| **Tillage** | Conventional | No-till | Conventional | |
| **Rotation** | Corn-Soybeans | Oat-Soybean | Corn-Soybeans | |
| **Experimental Design** | Split Plot Design | RCBD | Split Plot Design | RCBD |
| **Planting Date** | May 15$^{\dagger}$,<br>June 4 | May 17 | June 3,<br>June 13 | June 8 |
| **Pre plant application** | May 15,<br>June 4 | May 17 | June 3,<br>June 13 | June 8 |
| **V4 application** | June 18<br>July 9 | June 19 | July 8<br>July 15 | July 15 |
| **R3 application** | July13<br>Aug. 8 | July 10 | July 25<br>Aug 7 | July 25 |
| **MRML** | July 9<br>Aug. 1 | July 10 | July 18<br>July 26 | July 18 |
| **R5 Biomass** | Aug. 10, Aug. 13, Aug. 27 | Aug. 6 | Aug. 13,<br>Aug. 20, 22 | Aug. 15, 19 |
| **R6 Biomass** | Aug.31, Sep.7,<br>Sep. 14 | Aug. 20, 23 | Aug. 29, Sep.10,<br>Aug. 29, Sep.10 | Sep. 3, 5 |
| **R7 Biomass** | Sep. 11, 17,<br>Sep. 24, Oct. 1 | Sep.5,12 | Sep. 23,Oct. 4,<br>Sep.23, Oct. 4 | Sep. 23, 30 |
| **R8 Biomass** | Oct. 15 | Oct. 5 | Oct. 9,<br>Oct. 15, 16 | Oct. 11 |
| **Harvest Date** | Oct. 19-20 | Oct. 18 | Oct. 20 | Oct. 18 |

$^{\dagger}$ multiple dates in the line for a field activity indicate that the MG1 and MG2 varieties have been sampled separately on the days indicated for all experimental site, while different date in different lines for a field activity indicate the sampling time differences for the different planting dates at the Brookings site.

**Table 3-2. Pre plant field characteristics of soil pH, organic matter (OM), and soil nitrate-N ($NO_3^-$-N), and sulfate-S ($SO_4^{2-}$-S) concentration in the 0-15cm and 15-60cm soil zones in 2018 and 2019 near Brookings and Beresford, SD.**

| | Soil depth (cm) | pH | OM (%) | $NO_3^-$-N (ppm) | $SO_4^{2-}$-S (ppm) |
|---|---|---|---|---|---|
| **2018 Brookings** | 0-15 | 6.13 ± 0.53 | 4.73 ± 0.57 | 19.25 ± 4.3 | 12.25 ± 1.69 |
| | 15-60 | 6.25 ± 0.12 | 3.63 ± 0.25 | 7.75 ± 0.41 | 8.50 ± 0.57 |
| **2018 SERF** | 0-15 | 6.63 ± 0.79 | 3.40 ± 0.57 | 7.50 ± 2.52 | 8.00 ± 2 .16 |
| | 15-60 | 7.18 ± 0.64 | 2.98 ± 0.52 | 5.50 ± 1.91 | 12.00 ± 3.74 |
| **2019 Brookings** | 0-15 | 6.53 ± 0.24 | 3.95 ± 0.17 | 3.25 ± 1.26 | 3.00 ± 0.00 |
| | 15-60 | 7.25 ± 0.31 | 2.80 ± 0.47 | 2.75 ± 0.50 | 2.50 ± 0.58 |
| **2019 SERF** | 0-15 | 6.88 ± 0.70 | 2.95 ± 0.31 | 6.00 ± 2.45 | 5.25 ± 1.50 |
| | 15-60 | 7.48 ± 0.56 | 2.60 ± 0.36 | 8.00 ± 4.32 | 6.50 ± 4.36 |

**Table 3-3. Analysis of Variance (ANOVA) significance levels of nitrogen and sulfur application, year, location, and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in eastern SD in 2018 and 2019.**

| | Plant Stand | | Grain Yield | Grain Protein concentration | Grain Oil concentration |
|---|---|---|---|---|---|
| | Early | Late | | | |
| **Year** | *0.08* | **<.0001** | **<.0001** | **<.0001** | **0.03** |
| **Location** | **<.0001** | 0.18 | **<.0001** | **<.0001** | **<.0001** |
| **MG** | **<.0001** | **<.0001** | *0.07* | **<.0001** | **<.0001** |
| **Treatment** | 0.67 | 0.26 | 0.70 | 0.15 | **<.0001** |
| **Year*Location** | 0.23 | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Year*MG** | 0.27 | 0.15 | 0.24 | **0.04** | **<.0001** |
| **Location*MG** | 0.16 | *0.05* | **0.0005** | **<.0001** | **<.0001** |
| **Year* Treatment** | 0.74 | 0.68 | 0.14 | *0.06* | 0.64 |
| **Location* Treatment** | 0.37 | 0.69 | 0.79 | 0.52 | 0.31 |
| **MG* Treatment** | 0.20 | 0.64 | 0.81 | 0.73 | 0.95 |
| **Year*Location*MG** | 0.14 | **0.03** | 0.84 | 0.65 | **0.01** |
| **Year*Location* Treatment** | 0.68 | 0.89 | 0.79 | 0.42 | 0.97 |
| **Year*MG* Treatment** | 0.17 | 0.29 | 0.79 | 0.75 | 0.50 |
| **Location*MG* Treatment** | 0.61 | 0.50 | 0.70 | 0.96 | 0.85 |
| **Year*Location*MG*Treatment** | 0.37 | 0.40 | 0.83 | 0.39 | 0.40 |

**Table 3-4. Main treatment effect on early season and final plant stands in 2018 and 2019 near Brookings and Beresford, SD**

| | Early Stand | Final Stand | Yield | Protein | Oil |
|---|---|---|---|---|---|
| | (plants ha$^{-1}$) | | Mg ha$^{-1}$ | % | |
| **UTC**[†] | 245,600 | 244,900 | 4.00 | 34.74 | 18.22ab[††] |
| **Pre** | 257,700 | 240,900 | 4.01 | 34.82 | 18.04b |
| **V4** | 250,800 | 233,800 | 3.98 | 34.71 | 18.13ab |
| **R3** | 248,300 | 230,800 | 4.09 | 34.87 | 18.13ab |
| **V4+R3** | 252,300 | 241,900 | 4.05 | 34.67 | 18.22a |
| | | | | | |
| **MG1** | 263,200b | 246,900a | 4.09b | 34.6b | 18.0 |
| **MG2** | 275,800a | 237,200b | 4.33a | 35.0a | 18.3 |
| | | | | | |
| **2018** | 255,500b[†††] | 221,200b | 4.54a | 35.4a | 18.2 |
| **2019** | 283,400a | 255,700a | 3.53b | 34.1b | 18.1 |
| | | | | | |
| **Brookings** | 263,400a | 235,500 | 3.73b | 34.9a | 17.9b |
| **SERF** | 238,500b | 241,400 | 4.40a | 34.7b | 18.3a |

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at both V4 and R3 growth stage

[††] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

[†††] different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

**Table 3-5. Nitrogen and Sulfur study; Year by location, and location by maturity group interaction effects on grain yield grain protein concentrations, and the year by location by maturity group interaction effects on grain oil concentration near Brookings, SD and Beresford, SD in 2018 and 2019.**

| Year | Location | Maturity group | Grain Yield (Mg $ha^{-1}$) | Protein | Oil |
|---|---|---|---|---|---|
| | | | | (%) | |
| **2018** | **Brookings** | | 4.10b | 35.96a | 17.83b |
| | **SERF** | | 4.97a | 34.91b | 18.51a |
| **2019** | **Brookings** | | 3.35c | 33.83c | 18.13b |
| | **SERF** | | 3.71bc | 34.42bc | 18.12b |
| | | | | | |
| | **Brookings** | **MG1** | 3.68b | 34.88a | 17.83c |
| | | **MG2** | 3.77b | 34.91a | 18.13b |
| | **SERF** | **MG1** | 4.49a | 34.31b | 18.25ab |
| | | **MG2** | 4.19a | 35.02a | 18.38a |
| | | | | | |
| **2018** | **Brookings** | **MG1** | | | 17.78b |
| | | **MG2** | | | 17.88b |
| | **SERF** | **MG1** | | | 18.66a |
| | | **MG2** | | | 18.36a |
| **2019** | **Brookings** | **MG1** | | | 17.88b |
| | | **MG2** | | | 18.38a |
| | **SERF** | **MG1** | | | 17.84b |
| | | **MG2** | | | 18.40a |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

**Table 3-6. Analysis of Variance (ANOVA) significance levels of foliar protection application, year, location, planting date (PDate), and maturity group (MG) main effects and their interactions on early-season and final plant stand, grain yield, and grain protein and oil concentrations in the planting date comparison near Brookings, SD in 2018 and 2019.**

| | **Plant Stand** | | **Grain Yield** | **Grain Protein concentration** | **Grain Oil concentration** |
|---|---|---|---|---|---|
| | **Early** | **Late** | | | |
| **Year** | **0.02** | **<.0001** | **0.0008** | **<.0001** | **0.0002** |
| **PDate** | **<.0001** | **0.003** | **<.0001** | 0.13 | **0.02** |
| **MG** | 0.13 | 0.19 | 0.14 | **0.003** | **<.0001** |
| **Treatment** | 0.49 | 0.48 | 0.48 | *0.07* | 0.13 |
| **Year*PDate** | **<.0001** | 0.83 | **<.0001** | **<.0001** | **0.005** |
| **Year*MG** | 0.59 | **0.002** | **0.007** | 0.48 | **<.0001** |
| **Year*Treatment** | **0.01** | 0.65 | **0.005** | **0.0003** | **<.0001** |
| **PDate*MG** | 0.55 | *0.10* | 0.14 | 0.31 | 0.99 |
| **PDate*Treatment** | 0.38 | *0.05* | 0.43 | 0.28 | 0.19 |
| **MG*Treatment** | 0.50 | 0.78 | 0.97 | 0.86 | 0.91 |
| **Year*Pdate*MG** | 0.31 | 0.59 | *0.05* | **0.04** | **0.04** |
| **Year*Pdate*Treatment** | 0.95 | 0.28 | 0.34 | 0.97 | 0.95 |
| **Year*MG*Treatment** | 0.30 | **0.007** | 0.51 | 0.66 | 0.50 |
| **PDate*MG*Treatment** | 0.11 | 0.46 | 0.46 | 0.90 | 0.98 |
| **Year*Pdate*MG*Treatment** | 0.48 | 0.75 | 0.54 | 0.53 | 0.55 |

**Table 3-7. Effect on early season and final plant stands in 2018 and 2019 near Brookings, SD**

| | | Stand counts Early | Final |
|---|---|---|---|
| | | (plants $ha^{-1}$) | |
| ***Year*** | | | |
| **2018** | | 273,900b† | 210,800b† |
| **2019** | | 283,300a | 269,800a |
| | | | |
| ***Planting date*** | | | |
| **Pdate 1** | | 263,500b | 235,400b |
| **Pdate 2** | | 293,800a | 245,200a |
| | | | |
| ***Year*Planting Date*** | | | |
| **2018** | Pdate 1 | 271,300bc†† | |
| | Pdate 2 | 276,500b | |
| **2019** | Pdate 1 | 255,700c | |
| | Pdate 2 | 311,000a | |
| ***Year*Treatment*** | | | |
| **2018** | UTC††† | 272,900bc | |
| | Pre | 275,000bc | |
| | V4 | 276,600bc | |
| | R3 | 275,200bc | |
| | V4+R3 | 269,800c | |
| **2019** | UTC††† | 271,700bc | |
| | Pre | 289,300a | |
| | V4 | 289,700a | |
| | R3 | 277,800b | |
| | V4+R3 | 288,100a | |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

†† different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

†††Fertilizer application timings are UTC, control; pre-plant, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at both V4 and R3 growth stage

**Table 3-8. Nitrogen and Sulfur application timing and planting date effect on grain yield, protein and oil concentrations in Brookings SD in 2018 and 2019.**

| | | Yield | Protein | Oil |
|---|---|---|---|---|
| | | Mg/ha$^{-1}$ | % | |
| ***Year x Treatment*** | | | | |
| **2018** | **UTC††** | 3.70a† | 35.8a | 18.0ab |
| | **Pre-plant** | 3.42ab | 36.0a | 17.9ab |
| | **V4** | 3.39ab | 35.8a | 18.0ab |
| | **R3** | 3.68a | 35.9a | 18.0ab |
| | **V4+R3** | 3.71a | 35.7a | 18.1a |
| **2019** | **UTC** | 3.25b | 34.2b | 17.8ab |
| | **Pre-plant** | 3.37ab | 34.0bc | 17.7b |
| | **V4** | 3.39ab | 33.8c | 17.9ab |
| | **R3** | 3.33ab | 34.2b | 17.9ab |
| | **V4+R3** | 3.40ab | 33.9bc | 17.9ab |
| ***Year*Pdate*** | | | | |
| **2018** | **PDate1** | 4.10a | 36.0a | 17.8b |
| | **PDate2** | 3.05c | 35.7b | 17.9b |
| **2019** | **PDate1** | 3.34b | 33.8d | 18.1a |
| | **PDate2** | 3.36b | 34.2c | 17.9b |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

††Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at both V4 and R3 growth stage

**Table 3-9 Analysis of Variance (ANOVA) significance levels of N and S application, year, location, and maturity group (MG) main effects and their interactions on biomass in eastern SD in 2018 and 2019.**

| | Leaves | Main Stem | Branches + Petioles | Pods; Seeds/ Pod Shells | Total Biomass |
|---|---|---|---|---|---|
| | | | **R5** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Location** | **0.003** | **<.0001** | 0.75 | 0.6 | **<.0007** |
| **MG** | 0.37 | 0.63 | **<.0001** | **<.0001** | 0.70 |
| **Treatment** | 0.39 | 0.8 | 0.19 | 0.66 | 0.69 |
| **Year*Location** | **0.002** | 0.41 | 0.21 | 0.18 | 0.65 |
| **Year*MG** | 0.25 | 0.22 | **0.03** | **<.0001** | 0.15 |
| **Location*MG** | 0.89 | 0.57 | *0.05* | **<.0001** | *0.10* |
| **Year* Treatment** | 0.87 | 0.61 | 0.94 | 0.42 | 0.77 |
| **Location* Treatment** | 0.6 | 0.41 | 0.62 | 0.99 | 0.28 |
| **MG* Treatment** | 0.28 | 0.34 | 0.5 | 0.53 | 0.41 |
| **Year*Location*MG** | 0.17 | *0.07* | 0.16 | **<.0001** | 0.72 |
| **Year*Location* Treatment** | 0.87 | 0.74 | 0.93 | 0.68 | 0.87 |
| **Year*MG* Treatment** | 0.71 | 0.74 | 0.88 | 0.79 | 0.65 |
| **Location*MG*Treatment** | 0.51 | 0.29 | 0.81 | 0.82 | 0.82 |
| **Year*Location*MG*Treatment** | 0.81 | 0.5 | 0.57 | 0.49 | 0.88 |
| | | | **R6** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Location** | **<.0001** | **<.0001** | **<.0001** | 0.35 | **<.0001** |
| **MG** | 0.87 | *0.1* | **0.03** | 0.27 | 0.54 |
| **Treatment** | 0.96 | 0.89 | 0.96 | 0.65 | 0.80 |
| **Year*Location** | **<.0001** | **<.0001** | **<.0001** | **0.03** | **<.0001** |
| **Year*MG** | *0.1* | 0.14 | 0.93 | **<.0001** | **0.003** |
| **Location*MG** | **0.003** | **0.02** | 0.85 | **0.008** | 0.57 |
| **Year* Treatment** | 0.63 | 0.35 | 0.75 | 0.79 | 0.31 |
| **Location* Treatment** | 0.16 | 0.25 | 0.34 | 0.67 | 0.30 |
| **MG* Treatment** | 0.65 | *0.05* | 0.82 | 0.4 | *0.06* |
| **Year*Location*MG** | 0.66 | **0.004** | 0.61 | *0.08* | 0.44 |
| **Year*Location* Treatment** | 0.13 | 0.14 | 0.13 | **0.04** | *0.06* |
| **Year*MG* Treatment** | 0.54 | *0.05* | 0.52 | 0.17 | 0.29 |
| **Location*MG*Treatment** | 0.78 | 0.54 | 0.7 | 0.99 | 0.95 |
| **Year*Location*MG*Treatment** | 0.55 | 0.87 | 0.57 | 0.58 | 0.60 |

Table 3-9 cont.

| | **Leaves** | **Main Stem** | **Branches + Petioles** | **Pods; Seeds/ Pod Shells** | **Total Biomass** |
|---|---|---|---|---|---|
| | | | **R7** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Location** | **0.01** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **MG** | **<.0001** | 0.15 | **<.0001** | 0.37 | 0.36 |
| **Treatment** | 0.77 | 0.45 | 0.11 | 0.26 | 0.85 |
| **Year*Location** | **<.0001** | **<.0001** | 0.55 | **<.0001** | 0.30 |
| **Year*MG** | **0.001** | **0.02** | **<.0001** | 0.79 | 0.29 |
| **Location*MG** | 0.69 | **<.0001** | **<.0001** | *0.06* | 0.12 |
| **Year* Treatment** | 0.51 | 0.64 | 0.42 | 0.45 | 0.86 |
| **Location* Treatment** | **<.0001** | 0.88 | *0.08* | 0.35 | 0.45 |
| **MG* Treatment** | 0.61 | 0.91 | 0.19 | 0.48 | 0.70 |
| **Year*Location*MG** | 0.6 | **<.0001** | **<.0001** | *0.05* | 0.46 |
| **Year*Location* Treatment** | *0.1* | 0.74 | *0.07* | 0.46 | 0.86 |
| **Year*MG* Treatment** | 0.98 | 0.91 | 0.37 | 0.34 | 0.85 |
| **Location*MG*Treatment** | 0.19 | 0.39 | *0.08* | 0.66 | 0.20 |
| **Year*Location*MG*Treatment** | *0.06* | 0.55 | *0.1* | 0.45 | 0.24 |

**Table 3-10. Effect of Nitrogen and Sulfur applications, on R5, R6, and R7 biomass in eastern SD in 2018 and 2019.**

| | | Leaves | Main Stem | Branches and Petioles | Pods; Seeds/Pod Shells | Total Biomass |
|---|---|---|---|---|---|---|
| | | | (kg ha$^{-1}$) | | | |
| **R5** | UTC† | 1902 | 1558 | 1438 | 1370 | 6268 |
| | Preplant | 1913 | 1551 | 1474 | 1284 | 6222 |
| | V4 | 1861 | 1514 | 1400 | 1236 | 6011 |
| | R3 | 1856 | 1516 | 1364 | 1300 | 6036 |
| | V4+R3 | 1860 | 1731 | 1421 | 1317 | 6329 |
| **R6** | UTC | 1799 | 1981 | 1718 | 4018 | 9516 |
| | Preplant | 1782 | 1926 | 1717 | 3693 | 9118 |
| | V4 | 1813 | 2002 | 1712 | 3859 | 9386 |
| | R3 | 1813 | 1967 | 1682 | 3974 | 9436 |
| | V4+R3 | 1818 | 1999 | 1780 | 3886 | 9483 |
| **R7** | UTC | 284 | 1341 | 819* | 3406/2186 | 8036 |
| | Preplant | 297 | 1290 | 842 | 3106/2207 | 7742 |
| | V4 | 303 | 1267 | 861 | 3251/2276 | 7958 |
| | R3 | 297 | 1259 | 767 | 3106/2221 | 7650 |
| | V4+R3 | 283 | 1203 | 795 | 3289/2218 | 7788 |

**Table 3-10 cont.**

| | | Leaves | Main Stem | Branches and Petioles | Pods; Seeds/Pod Shells | Total Biomass |
|---|---|---|---|---|---|---|
| | | (kg ha$^{-1}$) | | | | |
| ***Year* Location*** | | | | | | |
| **R5** | | | | | | |
| **2018** | Brookings | 2498a†† | 2394a | 1641a | 1735a | 10102a |
| | SERF | 2108b | 1725b | 1769a | 1615a | 7219b |
| **2019** | Brookings | 1595c | 1344bc | 1224b | 727b | 4892c |
| | SERF | 1600c | 875c | 1140b | 780b | 4397c |
| **R6** | | | | | | |
| **2018** | Brookings | 3014a | 3682a | 2637a | 5045a | 14889a |
| | SERF | 1996b | 1786b | 1736b | 4561a | 7219b |
| **2019** | Brookings | 1599b | 1333b | 1231b | 3022b | 7262b |
| | SERF | 1554b | 780b | 1231b | 3223b | 4397b |
| **R7** | | | | | | |
| **2018** | Brookings | 684a | 2227a | 990a | 4040b/1580c | 10438a |
| | SERF | 313b | 1637b | 1171a | 4617a/1956a | 11345a |
| **2019** | Brookings | 95c | 1008c | 596b | 3349c/1592c | 6632b |
| | SERF | 401b | 975c | 731b | 4329ab/1772b | 8086b |

† Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at both V4 and R3 growth stage

†† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

*Branches and petiole fractions at R7growth stage contained mainly branches, as petioles have fallen

**Table 3-11. Percentages of biomass plant weights in eastern SD in 2018 and 2019.**

| | | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods; Seed/ Pod Shells** | **Fallen Leaves** |
|---|---|---|---|---|---|---|
| **R5** | UTC | 0.33 | 0.25 | 0.24 | 0.19 | N/A |
| | Pre | 0.33 | 0.25 | 0.25 | 0.18 | N/A |
| | V4 | 0.34 | 0.23 | 0.25 | 0.18 | N/A |
| | R3 | 0.32 | 0.25 | 0.24 | 0.20 | N/A |
| | V4+R3 | 0.32 | 0.24 | 0.24 | 0.20 | N/A |
| **R6** | UTC | 0.21 | 0.18 | 0.18 | 0.39 | 0.04 |
| | Pre | 0.23 | 0.20 | 0.16 | 0.38 | 0.07 |
| | V4 | 0.21 | 0.19 | 0.17 | 0.40 | 0.04 |
| | R3 | 0.20 | 0.18 | 0.17 | 0.39 | 0.04 |
| | V4+R3 | 0.20 | 0.19 | 0.17 | 0.39 | 0.04 |
| **R7** | UTC | 0.04 | 0.16 | 0.09 | 0.36/0.17 | 0.13 |
| | Pre | 0.06 | 0.17 | 0.11 | 0.37/0.17 | 0.15 |
| | V4 | 0.04 | 0.15 | 0.10 | 0.35/0.17 | 0.13 |
| | R3 | 0.04 | 0.15 | 0.07 | 0.39/0.17 | 0.14 |
| | V4+R3 | 0.04 | 0.15 | 0.10 | 0.32/0.17 | 0.14 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at both V4 and R3 growth stage

**Table 3-12. Analysis of Variance (ANOVA) significance levels of N and S application, year, location, planting date (PDate), and maturity group (MG) main effects and their interactions on biomass concentrations in Brookings, SD in 2018 and 2019.**

| | Leaves | Main Stem | Branches + Petioles | Pods; Seeds/ Pod Shells | Total Biomass |
|---|---|---|---|---|---|
| | | | **R5** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **<.0001** | **<.0001** |
| **Pdate** | **<.0001** | **<.0001** | **0.003** | **<.0001** | 0.32 |
| **MG** | 0.35 | 0.75 | **<.0001** | **<.0001** | 0.70 |
| **Treatment** | 0.94 | 0.81 | 0.96 | 0.60 | 0.96 |
| **Year*Pdate** | **<.0001** | **<.0001** | **<.0001** | **0.03** | **0.01** |
| **Year*MG** | 0.87 | **0.007** | 0.20 | **0.004** | 0.21 |
| **Year*Treatment** | 0.85 | 0.33 | 0.66 | **<.0001** | 0.15 |
| **Pdate*MG** | 0.84 | 0.36 | 0.90 | 0.70 | 0.76 |
| **Pdate*Treatment** | 0.44 | 0.44 | 0.65 | 0.45 | 0.61 |
| **MG*Treatment** | 0.19 | 0.89 | 0.28 | 0.30 | 0.39 |
| **Year*Pdate*MG** | 0.94 | 0.79 | 0.74 | 0.02 | 0.64 |
| **Year*Pdate*Treatment** | 0.85 | 0.63 | 0.32 | 0.93 | 0.70 |
| **Year*MG*Treatment** | 0.80 | 0.75 | 0.80 | 0.48 | 0.69 |
| **Pdate*MG*Treatment** | 0.63 | 0.28 | 0.81 | 0.64 | 0.49 |
| **Year*Pdate*MG*Treatment** | 0.75 | 0.85 | 0.42 | 0.82 | 0.78 |
| | | | **R6** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | **0.007** | **<.0001** |
| **Pdate** | **<.0001** | **<.0001** | **<.0001** | 0.98 | **<.0001** |
| **MG** | **0.04** | 0.22 | **<.0001** | 0.96 | 0.35 |
| **Treatment** | 0.64 | 0.45 | 0.65 | 0.96 | 0.84 |
| **Year*Pdate** | **<.0001** | **<.0001** | 0.48 | **<.0001** | **<.0001** |
| **Year*MG** | 0.05 | **<.0001** | 0.50 | 0.33 | 0.15 |
| **Year*Treatment** | **<.0001** | **0.003** | 0.06 | 0.10 | 0.73 |
| **Pdate*MG** | 0.09 | 0.03 | 0.51 | 0.33 | *0.08* |
| **Pdate*Treatment** | 0.57 | 0.75 | 0.90 | 0.97 | 0.97 |
| **MG*Treatment** | 0.94 | 0.11 | 0.85 | 0.53 | 0.45 |
| **Year*Pdate*MG** | 0.81 | 0.57 | 0.78 | 0.62 | 0.70 |
| **Year*Pdate*Treatment** | 0.21 | 0.71 | 0.26 | 0.93 | 0.65 |
| **Year*MG*Treatment** | 0.77 | **0.02** | 0.99 | 0.90 | 0.67 |
| **Pdate*MG*Treatment** | 0.49 | 0.58 | 0.14 | 0.62 | 0.65 |
| **Year*Pdate*MG*Treatment** | 0.99 | 0.90 | 0.79 | 0.83 | 0.99 |

Table 3-12 cont.

| | **Leaves** | **Main Stem** | **Branches + Petioles** | **Pods; Seeds/ Pod Shells** | **Total Biomass** |
|---|---|---|---|---|---|
| | | | **R7** | | |
| **Year** | **<.0001** | **<.0001** | **<.0001** | *0.05*/**0.01** | **<.0001** |
| **Pdate** | **0.003** | **<.0001** | **0.02** | **0.01/0.09** | **0.002** |
| **MG** | **<.0001** | **0.02** | **<.0001** | 0.37/0.21 | 0.65 |
| **Treatment** | 0.07 | 0.60 | 0.20 | 0.12/0.28 | 0.38 |
| **Year*Pdate** | **<.0001** | **<.0001** | **<.0001** | 0.11/**<.0001** | **<.0001** |
| **Year*MG** | **<.0001** | **<.0001** | **0.02** | 0.06/0.62 | 0.17 |
| **Year*Treatment** | **<.0001** | **0.03** | **<.0001** | 0.68/0.16 | 0.70 |
| **Pdate*MG** | 0.11 | 0.41 | *0.07* | 0.84/0.63 | 0.81 |
| **Pdate*Treatment** | *0.05* | 0.96 | 0.17 | *0.05*/0.49 | 0.15 |
| **MG*Treatment** | 0.27 | 0.71 | 0.28 | 0.53/0.42 | 0.53 |
| **Year*Pdate*MG** | **0.004** | 0.11 | **<.0001** | *0.06*/0.32 | 0.48 |
| **Year*Pdate*Treatment** | *0.06* | 0.93 | 0.46 | 0.58/0.19 | 0.59 |
| **Year*MG*Treatment** | 0.64 | 0.74 | 0.86 | 0.98/0.84 | 0.99 |
| **Pdate*MG*Treatment** | 0.28 | 0.74 | 0.35 | 0.45/0.45 | 0.46 |
| **Year*Pdate*MG*Treatment** | 0.50 | 0.92 | 0.42 | 0.25/0.62 | 0.47 |

**Table 3-13. Effect of Nitrogen and Sulfur applications, on R5, R6, R7 biomass in Brookings, SD in 2018 and 2019.**

| | | Leaves | Main Stem | Branches and Petioles | Pods; Seeds/ Pod Shells | Total Biomass |
|---|---|---|---|---|---|---|
| | | | | (kg ha$^{-1}$) | | |
| **R5** | UTC | 1902 | 1558 | 1438 | 1370 | 6268 |
| | Preplant | 1913 | 1551 | 1474 | 1284 | 6222 |
| | V4 | 1861 | 1514 | 1400 | 1236 | 6011 |
| | R3 | 1856 | 1516 | 1364 | 1300 | 6036 |
| | V4+R3 | 1860 | 1731 | 1421 | 1317 | 6329 |
| **R6** | UTC | 1799 | 1981 | 1718 | 4018 | 9516 |
| | Preplant | 1782 | 1926 | 1717 | 3693 | 9118 |
| | V4 | 1813 | 2002 | 1712 | 3859 | 9386 |
| | R3 | 1813 | 1967 | 1682 | 3974 | 9436 |
| | V4+R3 | 1818 | 1999 | 1780 | 3886 | 9483 |
| **R7** | UTC | 284 | 1341 | 819* | 3406/2186 | 8036 |
| | Preplant | 297 | 1290 | 842 | 3106/2207 | 7742 |
| | V4 | 303 | 1267 | 861 | 3251/2276 | 7958 |
| | R3 | 297 | 1259 | 767 | 3106/2221 | 7650 |
| | V4+R3 | 283 | 1203 | 795 | 3289/2218 | 7788 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

**Table 3-14. Percentages of biomass plant weights in Brookings, SD in 2018 and 2019 on two planting dates.**

| | | Leaves | Main Stem | Branches and Petioles | Pods; Seed/ Pod Shells | Fallen Leaves |
|---|---|---|---|---|---|---|
| **R5** | UTC | 0.30 | 0.26 | 0.22 | 0.20 | N/A |
| | Pre | 0.28 | 0.28 | 0.23 | 0.20 | N/A |
| | V4 | 0.31 | 0.24 | 0.23 | 0.20 | N/A |
| | R3 | 0.30 | 0.26 | 0.22 | 0.21 | N/A |
| | V4+R3 | 0.30 | 0.26 | 0.22 | 0.20 | N/A |
| **R6** | UTC | 0.18 | 0.22 | 0.18 | 0.39 | 0.05 |
| | Pre | 0.21 | 0.22 | 0.18 | 0.35 | 0.05 |
| | V4 | 0.18 | 0.21 | 0.17 | 0.39 | 0.05 |
| | R3 | 0.18 | 0.22 | 0.17 | 0.39 | 0.04 |
| | V4+R3 | 0.17 | 0.22 | 0.18 | 0.38 | 0.04 |
| **R7** | UTC | 0.03 | 0.18 | 0.08 | 0.34/0.18 | 0.11 |
| | Pre | 0.08 | 0.17 | 0.10 | 0.32/0.17 | 0.14 |
| | V4 | 0.05 | 0.17 | 0.10 | 0.33/0.17 | 0.11 |
| | R3 | 0.05 | 0.18 | 0.09 | 0.35/0.16 | 0.12 |
| | V4+R3 | 0.04 | 0.16 | 0.09 | 0.33/0.17 | 0.13 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

**Table 3-15. Nitrogen and Sulfur application effect on N, P, K, S, concentration in most recently matured leaves (MRML) tissue samples taken at R2 growth stage in eastern SD in 2018.**

| Year/Location | Treatment | N- | P | K | S |
|---|---|---|---|---|---|
| | | (ppm) | | | |
| **Brookings** | | 5.37 | 0.37 | 2.26a | 0.27b |
| **SERF** | | 5.42 | 0.36 | 2.06b | 0.30a |
| | **UTC** | 5.26c | 0.36 | 2.17 | 0.27b |
| | **Preplant** | 5.41ab | 0.37 | 2.12 | 0.29a |
| | **V4** | 5.45a | 0.36 | 2.11 | 0.29a |
| | **R3** | 5.35b | 0.37 | 2.22 | 0.28ab |
| | **V4+R3** | 5.49a | 0.37 | 2.16 | 0.29a |
| | *p*<F | | | | |
| | Location | 0.25 | 0.24 | **<.0001** | **<.0002** |
| | MG | 0.27 | 0.89 | 0.26 | 0.39 |
| | Treatment | *0.08* | 0.33 | 0.80 | *0.09* |
| | Location*MG | 0.43 | 0.96 | 0.79 | 0.96 |
| | Location* Treatment | 0.96 | 0.49 | 0.62 | 0.46 |
| | MG* Treatment | 0.62 | 0.91 | 0.96 | 0.54 |
| | Location*MG*Treatment | 0.47 | 0.62 | 0.53 | 0.98 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage

**Table 3-16. Nitrogen and Sulfur application effect on N, P, K, S, concentration in most recently matured leaves (MRML) tissue samples taken at the R2 growth stage in Brookings SD in 2018.**

| Year/Location | Treatment | N | P | K | S |
|---|---|---|---|---|---|
| | | | | ppm | |
| **Pdate 1** | | 5.36 | 0.37 | 2.26a | 0.27 |
| **Pdate 2** | | 5.36 | 0.38 | 2.19b | 0.27 |
| | | | | | |
| **UTC** | | 5.27 | 0.36 | 2.20 | 0.26b |
| **Pre-plant** | | 5.36 | 0.38 | 2.19 | 0.28a |
| **V4** | | 5.43 | 0.38 | 2.25 | 0.28a |
| **R3** | | 5.28 | 0.37 | 2.22 | 0.26b |
| **V4+R3** | | 5.47 | 0.37 | 2.25 | 0.28a |
| | | | | | |
| | *p*<F | | | | |
| | PDate | 0.87 | 0.38 | *0.06* | 0.30 |
| | MG | 0.35 | 0.52 | 0.40 | 0.68 |
| | Treatment | 0.17 | 0.12 | 0.93 | *0.09* |
| | PDate*MG | 0.20 | 0.46 | *0.06* | 0.15 |
| | PDate*Treatment | 0.50 | 0.20 | 0.41 | 0.53 |
| | MG*Treatment | 0.48 | 0.64 | 0.64 | 0.44 |
| | PDate*MG*Treatment | 0.65 | 0.39 | 0.20 | 0.91 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at pre-plant;V4, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at V4 growth stage; R3,11 kg S ha-1 and 44.8 kg N ha-1fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha-1 and 44.8 kg N ha-1 fertilizer application rate at both V4 and R3 growth stage.

**Table 3-17. Nitrogen and Sulfur application effect on N, K, S, in most recently matured leaves (MRML) tissue samples taken at the R2 growth stage in eastern SD in 2018.**

| | | Brookings/ SERF | | | PDates | |
|---|---|---|---|---|---|---|
| | | **N** | **S** | **K** | **S** | **K** |
| | | | (ppm) | | | |
| ***Location*** | | | | | | |
| **Brookings** | | | 0.27b | 2.22a | | |
| **SERF** | | | 0.30a | 2.05b | | |
| ***Planting Date*** | | | | | | |
| **Pdate1** | | | | | | 2.26a |
| **Pdate2** | | | | | | 2.19b |
| ***Planting Date* MG*** | | | | | | |
| **Pdate 1** | MG1 | | | | | 2.24a |
| | MG2 | | | | | 2.27a |
| **Pdate2** | MG1 | | | | | 2.24a |
| | MG2 | | | | | 2.13b |

† different lower-case letters indicate statistically different results within a column at p=0.10 confidence level

**Figure 3-1. Nitrogen and Sulfur applications, R5, R6, R7 biomass weights in eastern SD in 2018 and 2019.**

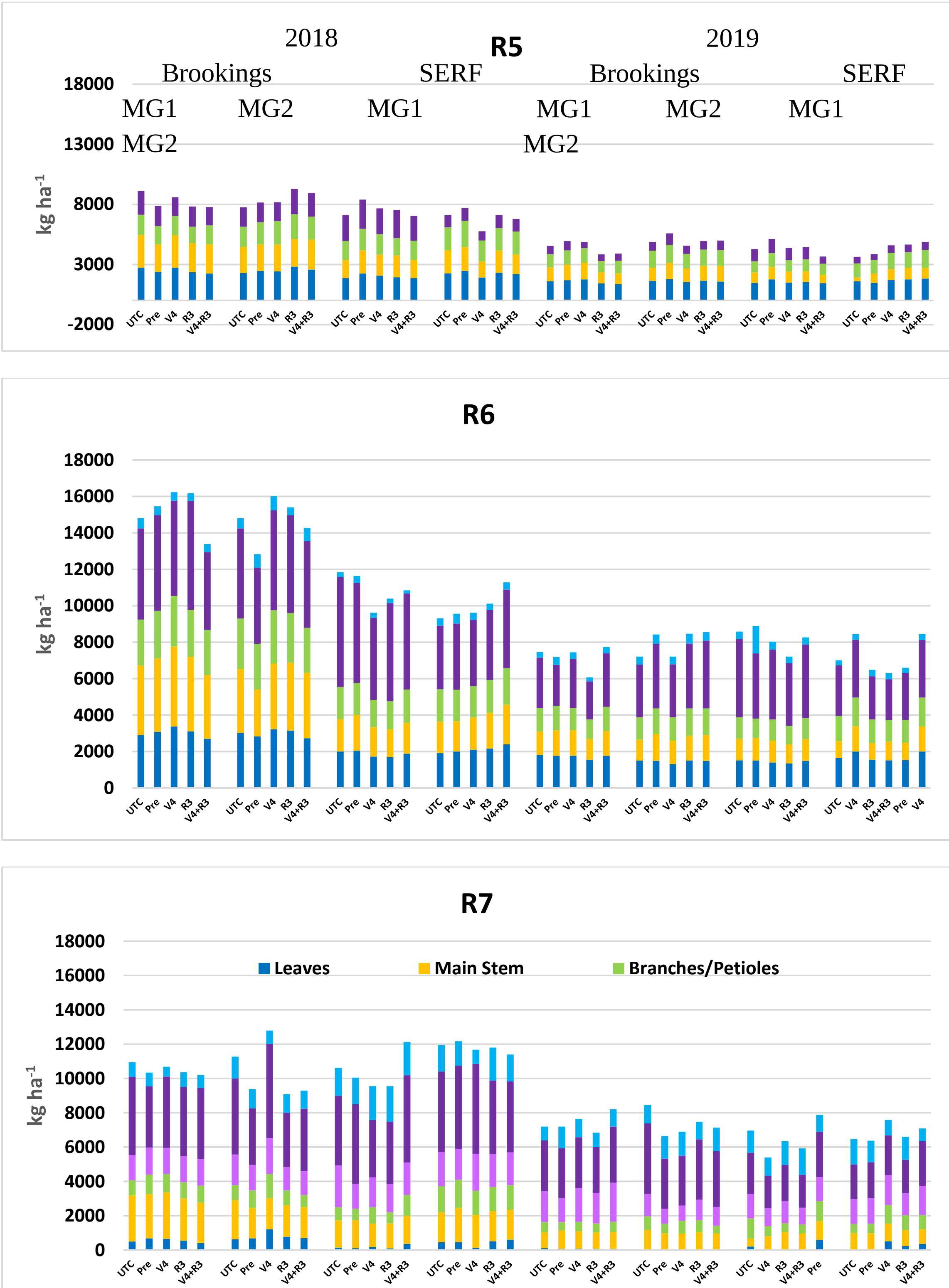
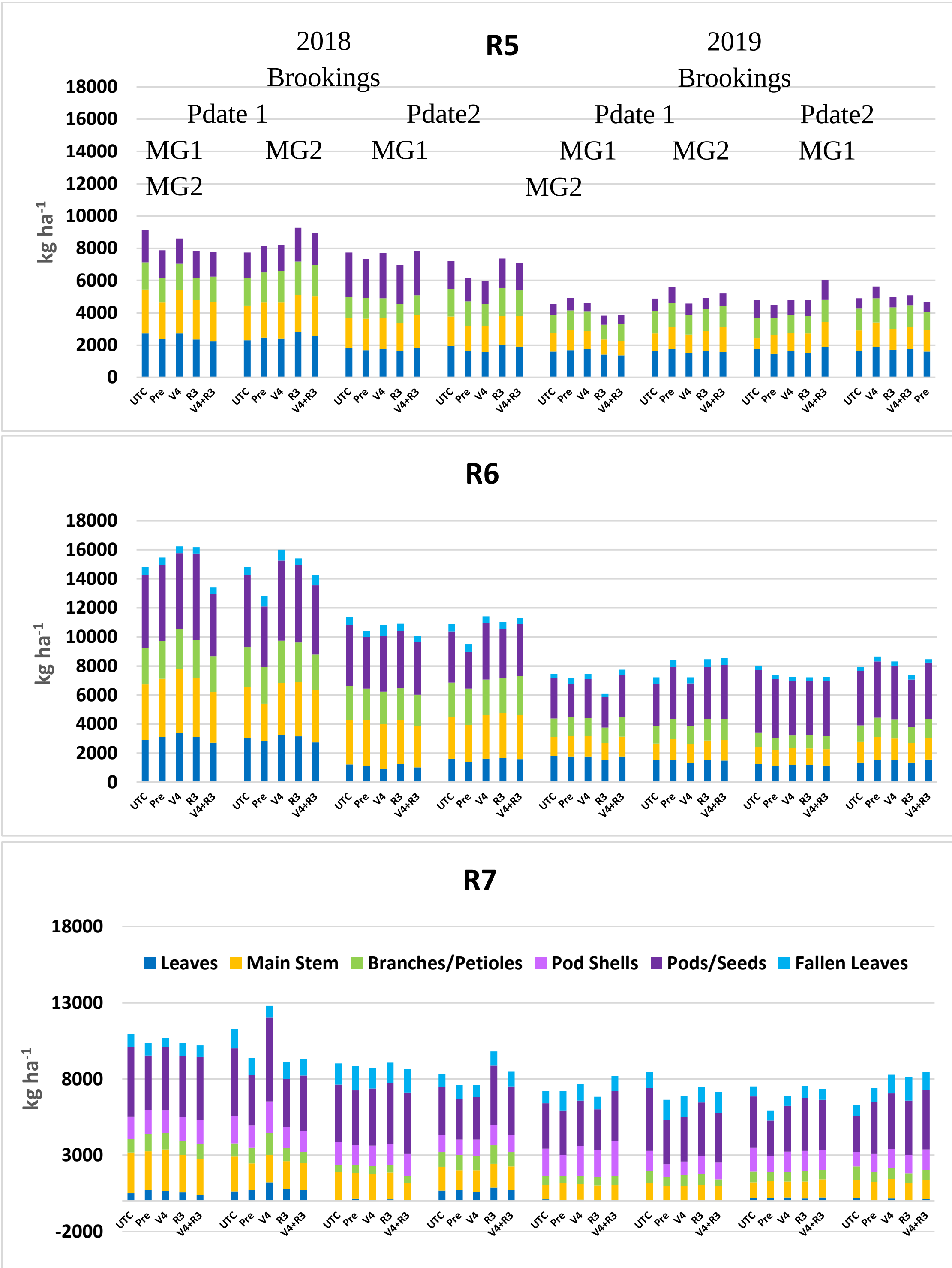


**Figure 3-2. Nitrogen and Sulfur applications, R5, R6, R7 biomass weights in Brookings SD in 2018 and 2019.**



# CHAPTER 4

## 4.1 OVERALL CONCLUSIONS

Overall, 2018 was not easily comparable to 2019 due to the delayed planting at Brookings and replanting at SERF in 2019 as a result of inclement weather conditions and the late spring. While comparing these two studies is difficult due to varieties used from different seed companies in the two studies and the varieties had somewhat different maturity lengths (1.0 vs 1.1 and 2.0 vs 2.4), there is a way to do so logically. When approaching a field, knowing some of its history is the key to finding what could increase the amount of protein in the plant. If a field is known to have low S, then applications of S may increase the amount of protein you receive while still maintaining or increasing yield. If a field is known to have a problem with a disease, then appropriate fungicide application could potentially help increase the amount of protein and grain yield at harvest.

The more products applied on a field, the more the production cost is. If there is not a problem in the field, there is not a reason to add unnecessary costs that will not help increase the grain quality or help benefit the amount of grain yield that is harvested. Only when there is a problem will benefits be seen when applying fungicide, insecticides, or fertilizer applications on both grain quality and grain yield.

The current study showed that the crop management practices evaluated had small influence on grain yield or seed composition during the two years of the project. We have experienced above normal precipitation amount during the early part of the growing season, and plenty of rainfall through the season which may influenced the results we have seen. With current soybean grain pricing structures at the elevators, there is no economic incentive for producing higher seed protein concentration while maximizing yield. It is not practical to convince farmers to produce a crop that has higher seed protein (or better seed composition) without providing some premium for their effort.

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# APPENDIX

**Table A-1. Foliar application effect on grain yield, protein and oil concentrations in eastern SD in 2018 and 2019.**

| Year/Location | MG | Treatment | Yield (Mg ha$^{-1}$) | Protein (%) | Oil (%) |
|---|---|---|---|---|---|
| **2018 Brookings** | MG1 | Control | 4.40 | 36.9a | 17.8b$^{\dagger}$ |
| | | Fungicide | 3.93 | 36.8a | 17.7b |
| | | Insecticide | 4.21 | 36.6a | 17.8b |
| | | Fungicide and Insecticide | 4.07 | 36.4ab | 18.0ab |
| | | FFI†† | 4.58 | 36.3ab | 18.1ab |
| | | Foliar Fertilizer | 4.79 | 36.8a | 17.8b |
| | MG2 | Control | 4.74 | 36.6a | 17.8b |
| | | Fungicide | 5.29 | 36.4ab | 18.0ab |
| | | Insecticide | 4.92 | 36.5ab | 17.9ab |
| | | Fungicide and Insecticide | 4.86 | 35.8b | 18.4a |
| | | FFI | 4.14 | 36.5ab | 18.0ab |
| | | Foliar Fertilizer | 4.90 | 36.4ab | 18.1ab |
| **2019 Brookings** | MG1 | Control | 3.36 | 34.9ab | 17.5b |
| | | Fungicide | 3.45 | 34.8ab | 17.3b |
| | | Insecticide | 3.32 | 34.8abc | 17.4b |
| | | Fungicide and Insecticide | 3.46 | 34.8abc | 17.4b |
| | | FFI | 3.51 | 34.8abc | 17.5b |
| | | Foliar Fertilizer | 3.27 | 34.9a | 17.5b |
| | MG2 | Control | 3.65 | 34.4abcd | 18.1a |
| | | Fungicide | 3.93 | 34.2cd | 18.4a |
| | | Insecticide | 3.80 | 34.3bcd | 18.2a |
| | | Fungicide and Insecticide | 3.82 | 34.3abcd | 18.2a |
| | | FFI | 4.02 | 34.6abcd | 18.1a |
| | | Foliar Fertilizer | 3.47 | 34.1d | 18.3a |

**Table A-1 cont.**

| **Year/Location** | MG | Treatment | Yield **(Mg ha$^{-1}$)** | Protein **(%)** | Oil **(%)** |
|---|---|---|---|---|---|
| **2019 SERF** | MG1 | Control | 3.64 | 34.9ab | 18.1b |
| | | Fungicide | 3.85 | 34.8ab | 17.9b |
| | | Insecticide | 3.66 | 34.8abc | 18.1b |
| | | Fungicide and Insecticide | 3.64 | 34.8abc | 18.0b |
| | | FFI | 3.77 | 34.8abc | 18.0b |
| | | Foliar Fertilizer | 3.69 | 34.9ab | 18.0b |
| | MG2 | Control | 3.92 | 34.4abcd | 18.1a |
| | | Fungicide | 4.24 | 35.1a | 18.1a |
| | | Insecticide | 4.30 | 34.3bcd | 18.2a |
| | | Fungicide and Insecticide | 4.28 | 34.3abcd | 18.3a |
| | | FFI | 4.15 | 34.6abcd | 18.2a |
| | | Foliar Fertilizer | 4.18 | 34.1d | 18.2a |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

†† Combination of foliar fertilizer, fungicides and insecticide application

**Table A-2. Foliar application effect on grain yield, protein and oil concentrations in Beresford, SD in 2018.**

| | | **Yield** Mg $ha^{-1}$ | **Protein** % | **Oil** % |
|---|---|---|---|---|
| ***Maturity Group*** | ***Foliar application treatment*** | | | |
| **MG1** | Control | 5.53 | 35.1 bc[†] | 18.5c[†] |
| | Fungicide | 5.62 | 34.7 c | 18.7a |
| | Insecticide | 5.51 | 34.9 c | 18.4c |
| | Fungicide and Insecticide | 5.64 | 34.9 c | 18.5bc |
| **MG2** | Control | 5.52 | 35.0 bc | 18.7a |
| | Fungicide | 5.91 | 35.2 ab | 18.9a |
| | Insecticide | 5.71 | 35.0 bc | 18.8a |
| | Fungicide and Insecticide | 5.93 | 35.5 a | 18.7ab |
| | *p*<F | | | |
| | Treatment | 0.80 | *0.06* | 0.31 |
| | MG | *0.01* | 0.21 | 0.11 |
| | Treatment x MG | 0.11 | 0.75 | 0.81 |

[†] different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

**Table A-3. Foliar application, percentage of weights compared to total on R5, R6, and R7.**

| | | | | | 2018 | | | | | 2019 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stage | **Year** | **Location** | **MG** | **Treatment** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods** | **Fallen Leaves** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods** | **Fallen** |
| **R5** | 2018 | Brookings | MG 1 | UTC | 0.36 | 0.26 | 0.19 | 0.2 | N/A | 0.37 | 0.24 | 0.24 | 0.15 | N/A |
| | | | | Fungicide | 0.32 | 0.3 | 0.19 | 0.16 | N/A | 0.35 | 0.25 | 0.22 | 0.19 | N/A |
| | | | | Insecticide | 0.3 | 0.33 | 0.19 | 0.18 | N/A | 0.36 | 0.24 | 0.24 | 0.16 | N/A |
| | | | | Fungicide and Insecticide | 0.31 | 0.32 | 0.18 | 0.19 | N/A | 0.34 | 0.26 | 0.23 | 0.17 | N/A |
| | | | | Foliar Fertilizer | 0.33 | 0.28 | 0.22 | 0.17 | N/A | 0.35 | 0.24 | 0.24 | 0.18 | N/A |
| | | | | FFI†† | 0.31 | 0.32 | 0.18 | 0.19 | N/A | 0.36 | 0.25 | 0.22 | 0.17 | N/A |
| | | | MG 2 | UTC | 0.3 | 0.27 | 0.2 | 0.23 | N/A | 0.32 | 0.22 | 0.26 | 0.19 | N/A |
| | | | | Fungicide | 0.3 | 0.24 | 0.23 | 0.24 | N/A | 0.34 | 0.21 | 0.27 | 0.18 | N/A |
| | | | | Insecticide | 0.29 | 0.28 | 0.21 | 0.22 | N/A | 0.32 | 0.23 | 0.27 | 0.18 | N/A |
| | | | | Fungicide and Insecticide | 0.3 | 0.21 | 0.22 | 0.23 | N/A | 0.34 | 0.21 | 0.28 | 0.17 | N/A |
| | | | | Foliar Fertilizer | 0.31 | 0.25 | 0.22 | 0.23 | N/A | 0.34 | 0.19 | 0.29 | 0.17 | N/A |
| | | | | FFI†† | 0.29 | 0.27 | 0.17 | 0.27 | N/A | 0.35 | 0.24 | 0.25 | 0.16 | N/A |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Pods | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SERF | MG 1 | UTC | 0.41 | 0.28 | 0.03 | 0.27 | N/A | 0.27 | 0.15 | 0.14 | 0.15 | N/A |
| | | | | Fungicide | 0.30 | 0.20 | 0.24 | 0.23 | N/A | 0.3 | 0.03 | 0.18 | 0.16 | N/A |
| | | | | Insecticide | 0.31 | 0.21 | 0.21 | 0.27 | N/A | 0.26 | 0.15 | 0.16 | 0.14 | N/A |
| | | | | Fungicide and Insecticide | 0.31 | 0.23 | 0.20 | 0.25 | N/A | 0.26 | 0.13 | 0.15 | 0.15 | N/A |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.28 | 0.14 | 0.16 | 0.14 | N/A |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.27 | 0.15 | 0.16 | 0.13 | N/A |
| | | | MG 2 | UTC | 0.34 | 0.23 | 0.24 | 0.19 | N/A | 0.28 | 0.15 | 0.16 | 0.15 | N/A |
| | | | | Fungicide | 0.35 | 0.19 | 0.27 | 0.19 | N/A | 0.28 | 0.15 | 0.17 | 0.14 | N/A |
| | | | | Insecticide | 0.35 | 0.24 | 0.24 | 0.17 | N/A | 0.27 | 0.15 | 0.17 | 0.14 | N/A |
| | | | | Fungicide and Insecticide | 0.34 | 0.22 | 0.25 | 0.20 | N/A | 0.27 | 0.16 | 0.18 | 0.12 | N/A |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.29 | 0.15 | 0.18 | 0.15 | N/A |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.27 | 0.15 | 0.16 | 0.14 | N/A |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Pods | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R6** | | Brookings | MG 1 | UTC | 0.18 | 0.26 | 0.12 | 0.41 | 0.03 | 0.19 | 0.17 | 0.15 | 0.46 | 0.04 |
| | | | | Fungicide | 0.18 | 0.22 | 0.12 | 0.44 | 0.05 | 0.21 | 0.16 | 0.17 | 0.44 | 0.03 |
| | | | | Insecticide | 0.17 | 0.25 | 0.10 | 0.42 | 0.06 | 0.18 | 0.17 | 0.15 | 0.46 | 0.04 |
| | | | | Fungicide and Insecticide | 0.16 | 0.25 | 0.90 | 0.44 | 0.06 | 0.22 | 0.11 | 0.18 | 0.47 | 0.02 |
| | | | | Foliar Fertilizer | 0.16 | 0.20 | 0.12 | 0.44 | 0.07 | 0.20 | 0.15 | 0.16 | 0.45 | 0.04 |
| | | | | FFI†† | 0.17 | 0.25 | 0.10 | 0.43 | 0.05 | 0.20 | 0.18 | 0.15 | 0.44 | 0.03 |
| | | | MG 2 | UTC | 0.12 | 0.20 | 0.10 | 0.54 | 0.03 | 0.18 | 0.16 | 0.15 | 0.48 | 0.02 |
| | | | | Fungicide | 0.16 | 0.19 | 0.09 | 0.54 | 0.03 | 0.20 | 0.15 | 0.15 | 0.47 | 0.02 |
| | | | | Insecticide | 0.12 | 0.23 | 0.09 | 0.52 | 0.04 | 0.18 | 0.17 | 0.15 | 0.48 | 0.03 |
| | | | | Fungicide and Insecticide | 0.13 | 0.21 | 0.10 | 0.51 | 0.05 | 0.11 | 0.19 | 0.18 | 0.5 | 0.02 |
| | | | | Foliar Fertilizer | 0.10 | 0.23 | 0.09 | 0.51 | 0.06 | 0.18 | 0.15 | 0.16 | 0.48 | 0.03 |
| | | | | FFI†† | 0.12 | 0.24 | 0.08 | 0.52 | 0.03 | 0.2 | 0.17 | 0.17 | 0.45 | 0.02 |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Pods | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | SERF | MG 1 | UTC | 0.18 | 0.17 | 0.14 | 0.48 | 0.02 | 0.22 | 0.16 | 0.13 | 0.46 | 0.02 |
| | | | | Fungicide | 0.18 | 0.18 | 0.15 | 0.48 | 0.01 | 0.27 | 0.16 | 0.14 | 0.46 | 0.02 |
| | | | | Insecticide | 0.18 | 0.18 | 0.15 | 0.48 | 0.01 | 0.22 | 0.17 | 0.1 | 0.5 | 0.02 |
| | | | | Fungicide and Insecticide | 0.18 | 0.17 | 0.15 | 0.48 | 0.01 | 0.21 | 0.16 | 0.15 | 0.47 | 0.01 |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.22 | 0.16 | 0.15 | 0.46 | 0.02 |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.21 | 0.15 | 0.14 | 0.5 | 0.01 |
| | | | MG 2 | UTC | 0.19 | 0.16 | 0.16 | 0.46 | 0.03 | 0.22 | 0.15 | 0.16 | 0.44 | 0.03 |
| | | | | Fungicide | 0.20 | 0.16 | 0.16 | 0.45 | 0.03 | 0.24 | 0.15 | 0.17 | 0.42 | 0.02 |
| | | | | Insecticide | 0.18 | 0.18 | 0.14 | 0.47 | 0.03 | 0.23 | 0.16 | 0.14 | 0.45 | 0.02 |
| | | | | Fungicide and Insecticide | 0.19 | 0.17 | 0.15 | 0.46 | 0.03 | 0.23 | 0.15 | 0.16 | 0.45 | 0.01 |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.23 | 0.16 | 0.14 | 0.44 | 0.03 |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.27 | 0.17 | 0.19 | 0.36 | 0.02 |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Seeds/Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Seeds/Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R7** | | Brookings | MG 1 | UTC | 0.02 | 0.15 | 0.05 | 0.34/0.31 | 0.13 | 0.01 | 0.13 | 0.10 | 0.17/0.49 | 0.09 |
| | | | | Fungicide | 0.03 | 0.20 | 0.06 | 0.32/0.30 | 0.10 | 0.02 | 0.14 | 0.10 | 0.18/0.48 | 0.08 |
| | | | | Insecticide | 0.02 | 0.19 | 0.05 | 0.33/0.31 | 0.10 | 0.02 | 0.15 | 0.10 | 0.16/0.49 | 0.09 |
| | | | | Fungicide and Insecticide | 0.05 | 0.26 | 0.08 | 0.25/0.23 | 0.13 | 0.02 | 0.15 | 0.09 | 0.17/0.49 | 0.09 |
| | | | | Foliar Fertilizer | 0.03 | 0.16 | 0.08 | 0.31/0.35 | 0.08 | 0.11 | 0.08 | 0.08 | 0.18/0.46 | 0.09 |
| | | | | FFI†† | 0.01 | 0.18 | 0.05 | 0.34/0.32 | 0.09 | 0.04 | 0.15 | 0.08 | 0.18/0.46 | 0.07 |
| | | | MG 2 | UTC | 0.01 | 0.19 | 0.06 | 0.31/0.29 | 0.14 | N/A | 0.19 | 0.08 | 0.15/0.39 | 0.24 |
| | | | | Fungicide | 0.01 | 0.13 | 0.07 | 0.35/0.33 | 0.11 | N/A | 0.14 | 0.08 | 0.14/0.46 | 0.18 |
| | | | | Insecticide | 0.01 | 0.17 | 0.05 | 0.39/0.37 | 0.01 | N/A | 0.13 | 0.09 | 0.16/0.52 | 0.14 |
| | | | | Fungicide and Insecticide | 0.02 | 0.11 | 0.05 | 0.37/0.35 | 0.11 | 0.003 | 0.12 | 0.08 | 0.15/0.50 | 0.15 |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Seeds/Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Seeds/Pod Shell | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Foliar Fertilizer | 0.01 | 0.15 | 0.04 | 0.36/0.34 | 0.10 | N/A | 0.11 | 0.08 | 0.17/0.50 | 0.16 |
| | | | | FFI†† | 0.02 | 0.19 | 0.05 | 0.33/0.31 | 0.11 | N/A | 0.15 | 0.002 | 0.16/0.55 | 0.17 |
| | | SERF | MG 1 | UTC | 0.03 | 0.15 | 0.07 | 0.15/0.44 | 0.15 | 0.02 | 0.09 | 0.07 | 0.19/0.51 | 0.12 |
| | | | | Fungicide | 0.05 | 0.14 | 0.09 | 0.16/0.42 | 0.14 | 0.06 | 0.12 | 0.09 | 0.21/0.46 | 0.06 |
| | | | | Insecticide | 0.03 | 0.16 | 0.07 | 0.18/0.43 | 0.14 | 0.01 | 0.13 | 0.07 | 0.17/0.51 | 0.11 |
| | | | | Fungicide and Insecticide | 0.03 | 0.16 | 0.07 | 0.16/0.47 | 0.12 | 0.01 | 0.11 | 0.06 | 0.17/0.46 | 0.19 |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.02 | 0.12 | 0.06 | 0.19/0.48 | 0.12 |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.04 | 0.11 | 0.06 | 0.19/0.47 | 0.13 |
| | | | MG 2 | UTC | 0.02 | 0.16 | 0.09 | 0.17/0.50 | 0.07 | 0.03 | 0.09 | 0.07 | 0.18/0.51 | 0.11 |
| | | | | Fungicide | N/A | 0.14 | 0.09 | 0.17/0.52 | 0.07 | 0.05 | 0.11 | 0.09 | 0.16/0.52 | 0.08 |
| | | | | Insecticide | 0.03 | 0.14 | 0.09 | 0.17/0.51 | 0.06 | 0.03 | 0.12 | 0.08 | 0.17/0.51 | 0.11 |

Table A-3 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Seeds/ Pod Shells | 2018 Fallen Leaves and | 2019 Leaves | 2019 Main Stem | 2019 Branches and | 2019 Seeds/Pod Shell | 2019 Fallen Leaves and |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | Fungicide and Insecticide | 0.03 | 0.15 | 0.08 | 0.16/0.54 | 0.04 | 0.03 | 0.12 | 0.07 | 0.16/ 0.55 | 0.07 |
| | | | | Foliar Fertilizer | N/A | N/A | N/A | N/A | N/A | 0.01 | 0.08 | 0.02 | 0.19/ 0.56 | 0.14 |
| | | | | FFI†† | N/A | N/A | N/A | N/A | N/A | 0.05 | 0.11 | 0.09 | 0.16/ 0.52 | 0.07 |

†† Combination of foliar fertilizer, fungicides and insecticide application

**Table A-4. Nitrogen and Sulfur applications, effect on grain yield, protein and oil concentrations in eastern SD in 2018 and 2019.**

| | | Brookings | | | | SERF | |
|---|---|---|---|---|---|---|---|
| | | PDate 1 | | PDate 2 | | | |
| | | MG1 | MG2 | MG1 | MG2 | MG1 | MG2 |
| | | Grain Yield Mg $ha^{-1}$ | | | | | |
| **2018** | UTC | 4.08 | 4.10 | 3.69 | 2.91 | 5.23 | 4.81 |
| | Pre-plant | 3.98 | 4.33 | 3.22 | 2.16 | 5.11 | 4.72 |
| | V4 | 3.81 | 3.87 | 3.25 | 2.79 | 5.11 | 4.74 |
| | R3 | 4.16 | 4.37 | 3.25 | 2.93 | 5.14 | 4.77 |
| | V4+R3 | 4.38 | 3.97 | 3.52 | 2.98 | 5.22 | 4.88 |
| **2019** | UTC | 3.13 | 3.28 | 3.20 | 3.41 | 3.86 | 3.51 |
| | Pre-plant | 3.22 | 3.39 | 3.43 | 3.44 | 3.81 | 3.57 |
| | V4 | 3.43 | 3.49 | 3.30 | 3.38 | 3.92 | 3.65 |
| | R3 | 3.31 | 3.50 | 3.41 | 3.08 | 3.81 | 3.71 |
| | V4+R3 | 3.27 | 3.42 | 3.38 | 3.53 | 3.71 | 3.57 |

Table A-4 cont.

| | | Brookings | | | | SERF | |
|---|---|---|---|---|---|---|---|
| | | PDate1 | | PDate2 | | | |
| | | MG1 | MG2 | MG1 | MG2 | MG1 | MG2 |
| | | | | Protein (%) | | | |
| **2018** | UTC | 35.80 | 35.9 | 36.1 | 35.3 | 34.5 | 35.2 |
| | Pre-plant | 36.1 | 36.2 | 36.2 | 35.5 | 34.6 | 35.5 |
| | V4 | 36.0 | 35.9 | 35.8 | 35.3 | 34.5 | 35.5 |
| | R3 | 36.0 | 36.2 | 36.1 | 35.2 | 34.5 | 35.1 |
| | V4+R3 | 35.6 | 35.9 | 36.0 | 35.5 | 34.4 | 35.3 |
| **2019** | UTC | 34.1 | 33.8 | 34.6 | 34.4 | 34.1 | 34.6 |
| | Pre-plant | 33.6 | 33.9 | 34.3 | 34.2 | 34.1 | 34.7 |
| | V4 | 33.6 | 33.8 | 34.2 | 34.2 | 33.9 | 34.6 |
| | R3 | 34.2 | 33.9 | 34.5 | 34.2 | 34.3 | 34.9 |
| | V4+R3 | 33.8 | 33.6 | 34.3 | 34.1 | 34.2 | 34.8 |
| | | | | Oil (%) | | | |
| **2018** | UTC | 17.8 | 17.8 | 17.4 | 18.2 | 18.6 | 18.4 |
| | Pre-plant | 17.5 | 17.8 | 17.5 | 18.2 | 18.6 | 18.3 |
| | V4 | 17.8 | 18.0 | 17.5 | 18.3 | 18.7 | 18.3 |
| | R3 | 17.8 | 17.9 | 17.5 | 18.3 | 18.7 | 18.4 |
| | V4+R3 | 18.0 | 17.9 | 17.6 | 18.3 | 18.7 | 18.4 |
| **2019** | UTC | 17.7 | 18.5 | 17.4 | 18.3 | 17.8 | 18.4 |
| | Pre-plant | 17.7 | 18.2 | 17.4 | 18.4 | 17.8 | 18.4 |
| | V4 | 17.7 | 18.4 | 17.5 | 18.4 | 17.9 | 18.3 |
| | R3 | 17.7 | 18.4 | 17.4 | 18.4 | 17.8 | 18.4 |
| | V4+R3 | 17.8 | 18.6 | 17.4 | 18.4 | 17.9 | 18.5 |

† different lower-case letters indicate statistically different results within a column at p=0.05 confidence level

**Table A-5. R5, R6, R7 biomass by percentages of total weight in eastern SD in 2018 and 2019.**

| | | | | | | 2018 | | | | | 2019 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stage | **Year** | **Location** | **Pdate** | **MG** | **Treat-ment** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods** | **Fallen Leaves** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods** | **Fallen Leaves** |
| **R5** | 2018 | Brookings | Pdate 1 | MG 1 | UTC† | 0.30 | 0.30 | 0.18 | 0.22 | N/A | 0.37 | 0.24 | 0.24 | 0.15 | N/A |
| | | | | | Pre | 0.30 | 0.29 | 0.19 | 0.21 | N/A | 0.34 | 0.26 | 0.34 | 0.16 | N/A |
| | | | | | V4 | 0.32 | 0.32 | 0.19 | 0.18 | N/A | 0.35 | 0.23 | 0.24 | 0.15 | N/A |
| | | | | | R3 | 0.30 | 0.31 | 0.17 | 0.22 | N/A | 0.34 | 0.26 | 0.24 | 0.16 | N/A |
| | | | | | V4+R3 | 0.29 | 0.31 | 0.20 | 0.20 | N/A | 0.34 | 0.23 | 0.26 | 0.15 | N/A |
| | | | | MG 2 | UTC | 0.30 | 0.28 | 0.22 | 0.21 | N/A | 0.33 | 0.25 | 0.27 | 0.14 | N/A |
| | | | | | Pre | 0.30 | 0.27 | 0.23 | 0.20 | N/A | 0.35 | 0.23 | 0.27 | 0.15 | N/A |
| | | | | | V4 | 0.30 | 0.27 | 0.24 | 0.19 | N/A | 0.33 | 0.23 | 0.29 | 0.15 | N/A |
| | | | | | R3 | 0.30 | 0.25 | 0.22 | 0.23 | N/A | 0.32 | 0.24 | 0.27 | 0.17 | N/A |
| | | | | | V4+R3 | 0.29 | 0.28 | 0.21 | 0.22 | N/A | 0.33 | 0.25 | 0.26 | 0.15 | N/A |
| | | | Pdate 2 | MG 1 | UTC | 0.22 | 0.23 | 0.16 | 0.34 | 0.04 | 0.32 | 0.25 | 0.23 | 0.21 | N/A |
| | | | | | Pre | 0.22 | 0.25 | 0.17 | 0.31 | 0.05 | 0.23 | 0.46 | 0.19 | 0.12 | N/A |
| | | | | | V4 | 0.22 | 0.24 | 0.15 | 0.35 | 0.04 | 0.37 | 0.14 | 0.25 | 0.24 | N/A |
| | | | | | R3 | 0.23 | 0.24 | 0.16 | 0.33 | 0.04 | 0.33 | 0.26 | 0.23 | 0.18 | N/A |
| | | | | | V4+R3 | 0.22 | 0.25 | 0.14 | 0.33 | 0.05 | 0.34 | 0.24 | 0.24 | 0.19 | N/A |

Table A-5 cont.

| Stage | Year | Location | Pdate | MG | Treat-ment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods; Seeds/ Pod | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Pods; Seeds/ Pod | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MG 2 | UTC | 0.24 | 0.23 | 0.21 | 0.22 | 0.09 | 0.34 | 0.29 | 0.24 | 0.13 | N/A |
| | | | | | Pre | 0.24 | 0.23 | 0.23 | 0.21 | 0.09 | 0.31 | 0.26 | 0.23 | 0.20 | N/A |
| | | | | | V4 | 0.24 | 0.25 | 0.20 | 0.22 | 0.09 | 0.34 | 0.26 | 0.28 | 0.13 | N/A |
| | | | | | R3 | 0.26 | 0.23 | 0.22 | 0.23 | 0.05 | 0.34 | 0.29 | 0.24 | 0.13 | N/A |
| | | | | | V4+R3 | 0.25 | 0.25 | 0.21 | 0.22 | 0.06 | 0.33 | 0.27 | 0.27 | 0.13 | N/A |
| **R6** | | | Pdate 1 | MG 1 | UTC | 0.20 | 0.26 | 0.17 | 0.34 | 0.04 | 0.26 | 0.19 | 0.17 | 0.34 | 0.04 |
| | | | | | Pre | 0.24 | 0.31 | 0.02 | 0.40 | 0.04 | 0.37 | 0.20 | 0.29 | 0.14 | 0.06 |
| | | | | | V4 | 0.21 | 0.27 | 0.17 | 0.32 | 0.03 | 0.24 | 0.17 | 0.17 | 0.37 | 0.05 |
| | | | | | R3 | 0.19 | 0.25 | 0.16 | 0.37 | 0.03 | 0.25 | 0.19 | 0.19 | 0.31 | 0.04 |
| | | | | | V4+R3 | 0.20 | 0.26 | 0.18 | 0.32 | 0.03 | 0.23 | 0.19 | 0.16 | 0.36 | 0.05 |
| | | | | MG 2 | UTC | 0.20 | 0.24 | 0.19 | 0.33 | 0.04 | 0.18 | 0.16 | 0.18 | 0.42 | 0.06 |
| | | | | | Pre | 0.22 | 0.20 | 0.20 | 0.33 | 0.06 | 0.23 | 0.18 | 0.17 | 0.38 | 0.06 |
| | | | | | V4 | 0.20 | 0.22 | 0.18 | 0.34 | 0.05 | 0.21 | 0.16 | 0.17 | 0.40 | 0.06 |
| | | | | | R3 | 0.20 | 0.24 | 0.18 | 0.35 | 0.03 | 0.18 | 0.17 | 0.17 | 0.42 | 0.06 |
| | | | | | V4+R3 | 0.19 | 0.25 | 0.17 | 0.33 | 0.05 | 0.18 | 0.18 | 0.18 | 0.4 | 0.06 |

Table A-5 cont.

| Stage | Year | Location | Pdate | MG | Treat-ment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods; Seeds/ Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Pods; Seeds/Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | Pdate 2 | MG 1 | UTC | 0.11 | 0.27 | 0.21 | 0.37 | 0.05 | 0.17 | 0.15 | 0.13 | 0.52 | 0.04 |
| | | | | | Pre | 0.11 | 0.30 | 0.21 | 0.34 | 0.04 | 0.17 | 0.17 | 0.17 | 0.44 | 0.03 |
| | | | | | V4 | 0.09 | 0.28 | 0.21 | 0.36 | 0.07 | 0.15 | 0.14 | 0.12 | 0.54 | 0.04 |
| | | | | | R3 | 0.12 | 0.28 | 0.20 | 0.36 | 0.05 | 0.15 | 0.15 | 0.11 | 0.55 | 0.03 |
| | | | | | V4+R3 | 0.10 | 0.28 | 0.21 | 0.36 | 0.04 | 0.16 | 0.16 | 0.12 | 0.52 | 0.04 |
| | | | | MG 2 | UTC | 0.15 | 0.27 | 0.22 | 0.32 | 0.05 | 0.18 | 0.18 | 0.15 | 0.45 | 0.04 |
| | | | | | Pre | 0.15 | 0.27 | 0.26 | 0.27 | 0.06 | 0.16 | 0.15 | 0.12 | 0.53 | 0.04 |
| | | | | | V4 | 0.14 | 0.26 | 0.21 | 0.34 | 0.04 | 0.17 | 0.18 | 0.14 | 0.47 | 0.03 |
| | | | | | R3 | 0.15 | 0.28 | 0.22 | 0.31 | 0.04 | 0.17 | 0.19 | 0.15 | 0.45 | 0.04 |
| | | | | | V4+R3 | 0.14 | 0.27 | 0.24 | 0.32 | 0.04 | 0.18 | 0.18 | 0.16 | 0.44 | 0.03 |
| **R7** | | | Pdate 1 | MG 1 | UTC | 0.05 | 0.25 | 0.08 | 0.13/ 0.42 | 0.08 | 0.01 | 0.14 | 0.08 | 0.26/ 0.39 | 0.11 |
| | | | | | Pre | 0.07 | 0.25 | 0.11 | 0.15/ 0.34 | 0.08 | 0.01 | 0.15 | 0.07 | 0.19/ 0.40 | 0.17 |
| | | | | | V4 | 0.06 | 0.25 | 0.10 | 0.14/ 0.39 | 0.05 | 0.02 | 0.13 | 0.08 | 0.25/ 0.41 | 0.14 |
| | | | | | R3 | 0.05 | 0.24 | 0.09 | 0.15/ 0.39 | 0.08 | 0.01 | 0.15 | 0.07 | 0.19/ 0.40 | 0.12 |
| | | | | | V4+R3 | 0.04 | 0.23 | 0.10 | 0.15/ 0.40 | 0.08 | 0.01 | 0.13 | 0.07 | 0.26/ 0.39 | 0.12 |

Table A-5 cont.

| Stage | Year | Location | Pdate | MG | Treat-ment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Seeds/ Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Seeds/ Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MG 2 | UTC | 0.06 | 0.20 | 0.08 | 0.16/ 0.39 | 0.11 | N/A | 0.14 | 0.09 | 0.16/ 0.47 | 0.13 |
| | | | | | Pre | 0.07 | 0.19 | 0.10 | 0.16/ 0.35 | 0.12 | 0.01 | 0.12 | 0.07 | 0.28/ 0.40 | 0.2 |
| | | | | | V4 | 0.10 | 0.14 | 0.11 | 0.16/ 0.43 | 0.06 | N/A | 0.13 | 0.09 | 0.16/ 0.49 | 0.2 |
| | | | | | R3 | 0.09 | 0.20 | 0.09 | 0.15/ 0.35 | 0.12 | N/A | 0.15 | 0.08 | 0.13/ 0.44 | 0.14 |
| | | | | | V4+R 3 | 0.08 | 0.19 | 0.08 | 0.15/ 0.39 | 0.11 | N/A | 0.14 | 0.11 | 0.13/ 0.42 | 0.19 |
| | | | Pdate 2 | MG 1 | UTC | 0.01 | 0.21 | 0.05 | 0.16/ 0.42 | 0.15 | 0.02 | 0.15 | 0.09 | 0.18/ 0.46 | 0.09 |
| | | | | | Pre | 0.02 | 0.19 | 0.06 | 0.15/ 0.41 | 0.18 | N/A | 0.13 | 0.06 | 0.15/ 0.45 | 0.11 |
| | | | | | V4 | 0.01 | 0.19 | 0.06 | 0.16/ 0.43 | 0.15 | 0.03 | 0.14 | 0.09 | 0.21/ 0.45 | 0.09 |
| | | | | | R3 | 0.01 | 0.19 | 0.05 | 0.15/ 0.44 | 0.15 | 0.03 | 0.19 | 0.10 | 0.18/ 0.39 | 0.11 |
| | | | | | V4+R 3 | 0.01 | 0.13 | 0.05 | 0.17/ 0.46 | 0.18 | 0.03 | 0.15 | 0.09 | 0.19/ 0.44 | 0.1 |

Table A-5 cont.

| Stage | Year | Location | Pdate | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Seeds/ Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Seeds/ Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | | | MG 2 | UTC | 0.08 | 0.19 | 0.12 | 0.14/ 0.37 | 0.10 | 0.01 | 0.14 | 0.08 | 0.025 / 0.41 | 0.12 |
| | | | | | Pre | 0.09 | 0.17 | 0.13 | 0.13/ 0.35 | 0.12 | 0.03 | 0.16 | 0.08 | 0.18/ 0.45 | 0.12 |
| | | | | | V4 | 0.08 | 0.18 | 0.12 | 0.14/ 0.37 | 0.10 | 0.03 | 0.18 | 0.14 | 0.15/ 0.38 | 0.1 |
| | | | | | R3 | 0.09 | 0.16 | 0.12 | 0.14/ 0.40 | 0.10 | N/A | 0.17 | 0.09 | 0.16/ 0.46 | 0.11 |
| | | | | | V4+R 3 | 0.08 | 0.18 | 0.11 | 0.14/ 0.37 | 0.12 | 0.02 | 0.15 | 0.09 | 0.15/ 0.45 | 0.14 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at both V4 and R3 growth stage.

**Table A-6. R5, R6, R7 biomass by percentages of total weight in Bookings SD in 2018 and 2019.**

| | | | | | 2018 | | | | | 2019 | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Stage | **Year** | **Location** | **MG** | **Treatment** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods; Seeds/ Pod Shells** | **Fallen Leaves** | **Leaves** | **Main Stem** | **Branches and Petioles** | **Pods; Seeds/ Pod Shells** | **Fallen Leaves** |
| **R5** | 2018 | Brookings | MG1 | UTC† | 0.30 | 0.30 | 0.18 | 0.22 | N/A | 0.37 | 0.24 | 0.24 | 0.15 | N/A |
| | | | | Pre | 0.30 | 0.29 | 0.19 | 0.21 | N/A | 0.34 | 0.26 | 0.34 | 0.16 | N/A |
| | | | | V4 | 0.32 | 0.32 | 0.19 | 0.18 | N/A | 0.35 | 0.23 | 0.24 | 0.15 | N/A |
| | | | | R3 | 0.30 | 0.31 | 0.17 | 0.22 | N/A | 0.34 | 0.26 | 0.24 | 0.16 | N/A |
| | | | | V4+R3 | 0.29 | 0.31 | 0.20 | 0.20 | N/A | 0.34 | 0.23 | 0.26 | 0.15 | N/A |
| | | | MG2 | UTC | 0.30 | 0.28 | 0.22 | 0.21 | N/A | 0.33 | 0.25 | 0.27 | 0.14 | N/A |
| | | | | Pre | 0.30 | 0.27 | 0.23 | 0.20 | N/A | 0.35 | 0.23 | 0.27 | 0.15 | N/A |
| | | | | V4 | 0.30 | 0.27 | 0.24 | 0.19 | N/A | 0.33 | 0.23 | 0.29 | 0.15 | N/A |
| | | | | R3 | 0.30 | 0.25 | 0.22 | 0.23 | N/A | 0.32 | 0.24 | 0.27 | 0.17 | N/A |
| | | | | V4+R3 | 0.29 | 0.28 | 0.21 | 0.22 | N/A | 0.33 | 0.25 | 0.26 | 0.15 | N/A |
| | | SERF | MG1 | UTC | 0.26 | 0.21 | 0.22 | 0.30 | N/A | 0.35 | 0.20 | 0.22 | 0.23 | N/A |
| | | | | Pre | 0.27 | 0.23 | 0.21 | 0.29 | N/A | 0.35 | 0.27 | 0.26 | 0.12 | N/A |
| | | | | V4 | 0.27 | 0.23 | 0.22 | 0.28 | N/A | 0.34 | 0.21 | 0.22 | 0.24 | N/A |
| | | | | R3 | 0.26 | 0.24 | 0.19 | 0.31 | N/A | 0.34 | 0.21 | 0.23 | 0.23 | N/A |
| | | | | V4+R3 | 0.27 | 0.21 | 0.23 | 0.29 | N/A | 0.34 | 0.21 | 0.22 | 0.24 | N/A |
| | | | MG2 | UTC | 0.32 | 0.27 | 0.27 | 0.14 | N/A | 0.38 | 0.21 | 0.28 | 0.14 | N/A |
| | | | | Pre | 0.32 | 0.26 | 0.28 | 0.14 | N/A | 0.39 | 0.19 | 0.25 | 0.16 | N/A |
| | | | | V4 | 0.33 | 0.23 | 0.30 | 0.13 | N/A | 0.44 | 0.08 | 0.33 | 0.15 | N/A |
| | | | | R3 | 0.32 | 0.26 | 0.26 | 0.15 | N/A | 0.38 | 0.20 | 0.30 | 0.12 | N/A |
| | | | | V4+R3 | 0.32 | 0.24 | 0.28 | 0.15 | N/A | 0.37 | 0.20 | 0.29 | 0.14 | N/A |

Table A-6 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods; Seeds/ Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Pods; Seeds/ Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R6** | | Brookings | MG1 | UTC | 0.20 | 0.26 | 0.17 | 0.34 | 0.04 | 0.26 | 0.19 | 0.17 | 0.34 | 0.04 |
| | | | | Pre | 0.24 | 0.31 | 0.02 | 0.40 | 0.04 | 0.37 | 0.2 | 0.29 | 0.14 | 0.06 |
| | | | | V4 | 0.21 | 0.27 | 0.17 | 0.32 | 0.03 | 0.24 | 0.17 | 0.17 | 0.37 | 0.05 |
| | | | | R3 | 0.19 | 0.25 | 0.16 | 0.37 | 0.03 | 0.25 | 0.19 | 0.19 | 0.31 | 0.04 |
| | | | | V4+R3 | 0.20 | 0.26 | 0.18 | 0.32 | 0.03 | 0.23 | 0.19 | 0.16 | 0.36 | 0.05 |
| | | | MG2 | UTC | 0.20 | 0.24 | 0.19 | 0.33 | 0.04 | 0.18 | 0.16 | 0.18 | 0.42 | 0.06 |
| | | | | Pre | 0.22 | 0.20 | 0.20 | 0.33 | 0.06 | 0.23 | 0.18 | 0.17 | 0.38 | 0.06 |
| | | | | V4 | 0.20 | 0.22 | 0.18 | 0.34 | 0.05 | 0.21 | 0.16 | 0.17 | 0.40 | 0.06 |
| | | | | R3 | 0.20 | 0.24 | 0.18 | 0.35 | 0.03 | 0.18 | 0.17 | 0.17 | 0.42 | 0.06 |
| | | | | V4+R3 | 0.19 | 0.25 | 0.17 | 0.33 | 0.05 | 0.18 | 0.18 | 0.18 | 0.40 | 0.06 |
| | | SERF | MG1 | UTC | 0.17 | 0.15 | 0.17 | 0.51 | 0.02 | 0.19 | 0.14 | 0.14 | 0.47 | 0.05 |
| | | | | Pre | 0.18 | 0.17 | 0.15 | 0.47 | 0.03 | 0.19 | 0.17 | 0.16 | 0.46 | 0.17 |
| | | | | V4 | 0.18 | 0.17 | 0.16 | 0.47 | 0.03 | 0.18 | 0.14 | 0.14 | 0.50 | 0.05 |
| | | | | R3 | 0.17 | 0.14 | 0.16 | 0.52 | 0.02 | 0.17 | 0.14 | 0.12 | 0.40 | 0.05 |
| | | | | V4+R3 | 0.18 | 0.15 | 0.17 | 0.49 | 0.02 | 0.18 | 0.15 | 0.15 | 0.48 | 0.05 |
| | | | MG2 | UTC | 0.20 | 0.18 | 0.19 | 0.38 | 0.05 | 0.24 | 0.14 | 0.20 | 0.36 | 0.04 |
| | | | | Pre | 0.21 | 0.19 | 0.18 | 0.38 | 0.06 | 0.18 | 0.15 | 0.14 | 0.49 | 0.04 |
| | | | | V4 | 0.22 | 0.18 | 0.18 | 0.38 | 0.04 | 0.24 | 0.13 | 0.20 | 0.40 | 0.04 |
| | | | | R3 | 0.21 | 0.19 | 0.18 | 0.38 | 0.05 | 0.23 | 0.14 | 0.19 | 0.39 | 0.06 |
| | | | | V4+R3 | 0.22 | 0.19 | 0.17 | 0.39 | 0.03 | 0.24 | 0.16 | 0.19 | 0.37 | 0.06 |

Table A-6 cont.

| Stage | Year | Location | MG | Treatment | 2018 Leaves | 2018 Main Stem | 2018 Branches and Petioles | 2018 Pods; Seeds/ Pod Shells | 2018 Fallen Leaves | 2019 Leaves | 2019 Main Stem | 2019 Branches and Petioles | 2019 Pods; Seeds/ Pod Shells | 2019 Fallen Leaves |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| **R7** | | Brookings | MG1 | UTC | 0.05 | 0.25 | 0.08 | 0.13/0.42 | 0.08 | 0.01 | 0.14 | 0.08 | 0.26/0.39 | 0.11 |
| | | | | Pre | 0.07 | 0.25 | 0.11 | 0.15/0.34 | 0.08 | 0.24 | 0.16 | 0.19 | 0.35 | 0.17 |
| | | | | V4 | 0.06 | 0.25 | 0.10 | 0.14/0.39 | 0.05 | 0.02 | 0.13 | 0.08 | 0.25/0.41 | 0.14 |
| | | | | R3 | 0.05 | 0.24 | 0.09 | 0.15/0.39 | 0.08 | 0.01 | 0.15 | 0.07 | 0.19/0.40 | 0.12 |
| | | | | V4+R3 | 0.04 | 0.23 | 0.10 | 0.15/0.40 | 0.08 | 0.01 | 0.13 | 0.07 | 0.26/0.39 | 0.12 |
| | | | MG2 | UTC | 0.06 | 0.20 | 0.08 | 0.16/0.39 | 0.11 | N/A | 0.14 | 0.09 | 0.16/0.47 | 0.13 |
| | | | | Pre | 0.07 | 0.19 | 0.10 | 0.16/0.35 | 0.12 | 0.01 | 0.12 | 0.07 | 0.28/0.40 | 0.20 |
| | | | | V4 | 0.10 | 0.14 | 0.11 | 0.16/0.43 | 0.06 | N/A | 0.13 | 0.09 | 0.16/0.49 | 0.20 |
| | | | | R3 | 0.09 | 0.20 | 0.09 | 0.15/0.35 | 0.12 | N/A | 0.15 | 0.08 | 0.13/0.44 | 0.14 |
| | | | | V4+R3 | 0.08 | 0.19 | 0.08 | 0.15/0.39 | 0.11 | N/A | 0.14 | 0.11 | 0.13/0.42 | 0.19 |
| | | SERF | MG1 | UTC | 0.01 | 0.14 | 0.08 | 0.23/0.40 | 0.14 | N/A | 0.11 | 0.08 | 0.17/0.50 | 0.15 |
| | | | | Pre | 0.01 | 0.16 | 0.07 | 0.15/0.46 | 0.16 | 0.01 | 0.15 | 0.08 | 0.16/0.46 | 0.15 |
| | | | | V4 | 0.02 | 0.15 | 0.11 | 0.18/0.33 | 0.21 | N/A | 0.12 | 0.07 | 0.17/0.50 | 0.20 |
| | | | | R3 | 0.01 | 0.15 | 0.06 | 0.18/0.39 | 0.21 | N/A | 0.12 | 0.06 | 0.16/0.51 | 0.17 |
| | | | | V4+R3 | 0.03 | 0.15 | 0.11 | 0.15/0.39 | 0.17 | N/A | 0.14 | 0.07 | 0.14/0.45 | 0.21 |
| | | | MG2 | UTC | 0.04 | 0.15 | 0.13 | 0.16/0.37 | 0.14 | 0.05 | 0.11 | 0.11 | 0.17/0.50 | 0.16 |
| | | | | Pre | 0.04 | 0.17 | 0.15 | 0.16/0.35 | 0.13 | N/A | 0.13 | 0.07 | 0.13/0.45 | 0.17 |
| | | | | V4 | 0.01 | 0.15 | 0.12 | 0.19/0.46 | 0.07 | 0.03 | 0.11 | 0.11 | 0.17/0.42 | 0.10 |
| | | | | R3 | 0.04 | 0.14 | 0.12 | 0.17/0.37 | 0.16 | 0.03 | 0.06 | 0.04 | 0.20/0.50 | 0.15 |
| | | | | V4+R3 | 0.05 | 0.15 | 0.13 | 0.16/0.36 | 0.14 | 0.06 | 0.11 | 0.12 | 0.19/0.43 | 0.10 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at both V4 and R3 growth stage.

**Table A-7. MRML samples 2018**

| Year/Location | MG | Treatment | $N^-$ | P | K | S |
|---|---|---|---|---|---|---|
| | | | | (ppm) | | |
| **Brookings** | MG1 | UTC† | 5.17 | 0.35 | 2.20 | 0.25 |
| | | Pre-plant | 5.36 | 0.38 | 2.28 | 0.28 |
| | | V4 | 5.45 | 0.40 | 2.38 | 0.30 |
| | | R3 | 5.24 | 0.36 | 2.17 | 0.25 |
| | | V4+R3 | 5.39 | 0.36 | 2.17 | 0.27 |
| | MG2 | UTC | 5.28 | 0.35 | 2.26 | 0.26 |
| | | Pre-plant | 5.43 | 0.36 | 2.13 | 0.27 |
| | | V4 | 5.43 | 0.37 | 2.24 | 0.28 |
| | | R3 | 5.42 | 0.39 | 2.41 | 0.28 |
| | | V4+R3 | 5.48 | 0.36 | 2.31 | 0.28 |
| **SERF** | MG1 | UTC | 5.21 | 0.37 | 2.14 | 0.29 |
| | | Pre-plant | 5.42 | 0.37 | 2.06 | 0.31 |
| | | V4 | 5.43 | 0.34 | 1.87 | 0.29 |
| | | R3 | 5.43 | 0.36 | 2.08 | 0.28 |
| | | V4+R3 | 5.56 | 0.36 | 1.97 | 0.30 |
| | MG2 | UTC | 5.37 | 0.35 | 2.09 | 0.28 |
| | | Pre-plant | 5.42 | 0.36 | 2.02 | 0.29 |
| | | V4 | 5.50 | 0.33 | 1.93 | 0.30 |
| | | R3 | 5.31 | 0.38 | 2.22 | 0.29 |
| | | V4+R3 | 5.52 | 0.38 | 2.20 | 0.32 |

†Fertilizer application timings are UTC, control; pre-plant, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S $ha^{-1}$ and 44.8 kg N $ha^{-1}$ fertilizer application rate at both V4 and R3 growth stage.

**Table A-8. MRML samples between planting dates.**

| | Treatment | $N^-$ | P (ppm) | K | S |
|---|---|---|---|---|---|
| **Pdate1** | UTC | 5.17 | 0.35 | 2.2 | 0.25 |
| | Pre-plant | 5.36 | 0.38 | 2.28 | 0.28 |
| | V4 | 5.45 | 0.4 | 2.38 | 0.3 |
| | R3 | 5.22 | 0.36 | 2.17 | 0.25 |
| | V4+R3 | 5.39 | 0.36 | 2.17 | 0.28 |
| | UTC | 5.28 | 0.35 | 2.26 | 0.26 |
| | Pre-plant | 5.43 | 0.36 | 2.13 | 0.27 |
| | V4 | 5.43 | 0.37 | 2.24 | 0.29 |
| | R3 | 5.42 | 0.39 | 2.41 | 0.28 |
| | V4+R3 | 5.48 | 0.36 | 2.31 | 0.28 |
| **Pdate2** | UTC | 5.39 | 0.38 | 2.29 | 0.27 |
| | Pre-plant | 5.31 | 0.39 | 2.2 | 0.29 |
| | V4 | 5.42 | 0.37 | 2.22 | 0.27 |
| | R3 | 5.23 | 0.37 | 2.22 | 0.26 |
| | V4+R3 | 5.51 | 0.39 | 2.27 | 0.28 |
| | UTC | 5.25 | 0.35 | 2.05 | 0.26 |
| | Pre-plant | 5.35 | 0.39 | 2.14 | 0.27 |
| | V4 | 5.4 | 0.38 | 2.14 | 0.27 |
| | R3 | 5.26 | 0.36 | 2.09 | 0.26 |
| | V4+R3 | 5.5 | 0.38 | 2.24 | 0.27 |

[†]Fertilizer application timings are UTC, control; pre-plant, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at pre-plant;V4, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at V4 growth stage; R3,11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$fertilizer application rate at R3 growth stage;V4+R3, 11 kg S ha$^{-1}$ and 44.8 kg N ha$^{-1}$ fertilizer application rate at both V4 and R3 growth stage.